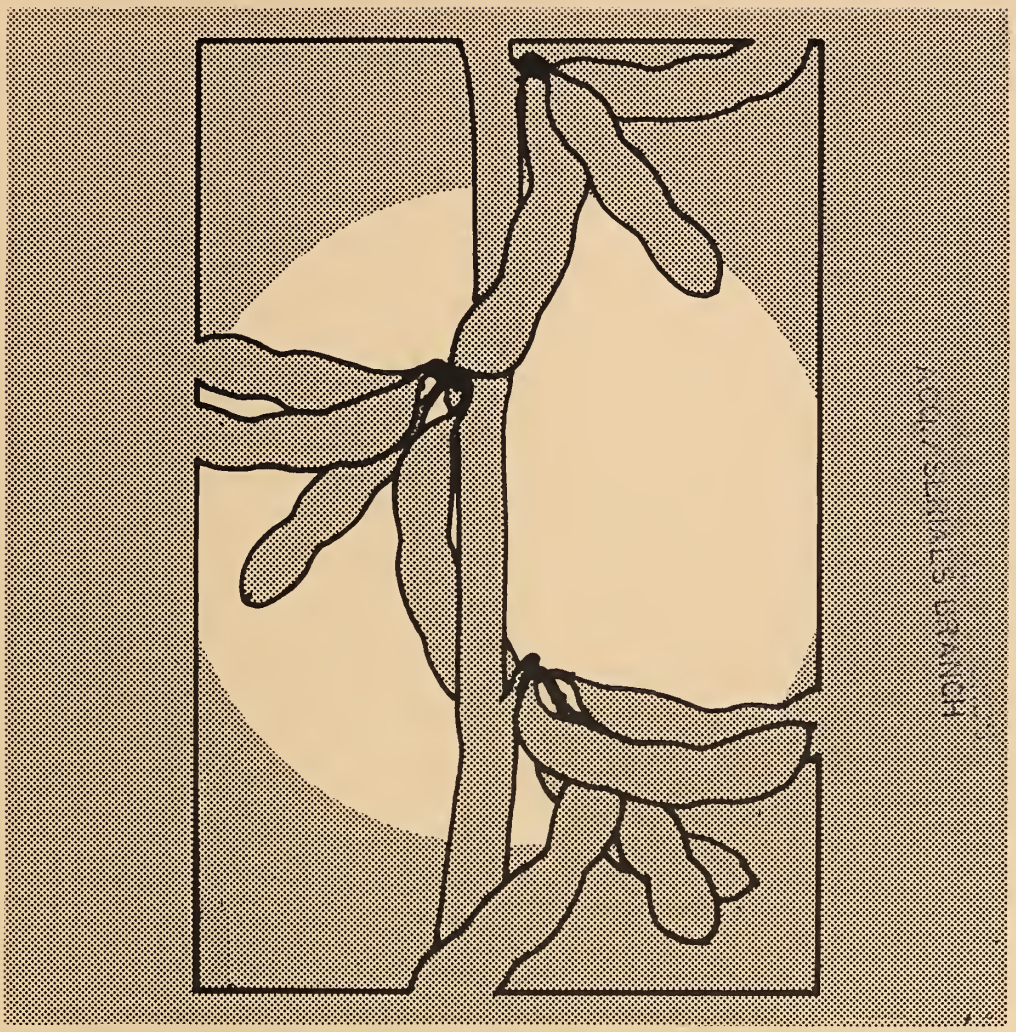
Soybean Genetics Newsletter



Volume 17 April 1990

The data presented here are nat ta be used in publicatians withaut the consent of the respective authars.

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FOREWORD

Volume 1 of the Soybean Genetics Newsletter was issued in April 1974, and was free. Due to escalating labor costs, printing and postage expenses, a subscription fee of $10 (US) per volume has been initiated this year, starting with Volume 17 (1990). We realize that this cost imposes a hardship on some soybean researchers. We sincerely regret the necessity of a subscription fee.

In the past, we have found it necessary to employ a graphic artist to reproduce some of the charts, graphs and diagrams included with articles submitted. We must insist that any charts, graphs or diagrams be photo-ready to be accepted. For Volume 18 (1991) we will return to the author any articles that contain unacceptable charts or diagrams, for their revision and correction.

The workers behind the scenes, who have made this volume possible, include Laurie Amberger, Holly Heer, Laura Sellner, and Fan Zhang. They have accomplished the library research, the reading, proofreading, and legwork necessary to prepare Volume 17.

R. G. Palmer, editor

SOYBEAN GENETICS COMMITTEE REPORT

FEBRUARY 1990

Minutes of the Meeting

The Soybean Genetics Committee met 26 Feb. 1990, in conjunction with the Soybean Breeder's Workshop in St. Louis, NO.

Committee members in attendance were G.R. Bowers, T.E. Devine, T.C. Kilen, B.A. McBlain, R.L. Nelson, and J.R. Wilcox. Also attending were P.B. Cregan, S.A. Mackenzie, and L.M. Mansur. S.A. Mackenzie and L.M. Mansur were elected to three-year terms, and T.E. Devine was elected Chairman for 1990-91. Current committee members and the year of membership expiration are listed:

T.E. Devine (1991) Chairman
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Procedures: As in the past, manuscripts concerning qualitative genetics interpretation, gene symbols and linkages should be sent to the chairman for review. The Committee will carry out the review process as indicated in the 1989 Genetics Newsletter 15:3.

Assignment/approval of gene symbols: If the gene symbols are being assigned in genetic studies where the material is from induced mutations, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Nelson is curator for all maturity groups. Application forms are available from the chairman, the curator, or in the Soybean Genetics Newsletter 16:6.

The committee will approve gene symbols only in cases where the relevant material is available for distribution to researchers. The committee encourages researchers not to assign any symbol when they are doing research with genes that will not be available. Genetic interpretation in publication is unaffected, in most cases. The purpose of an assigned gene symbol is constancy when others use the material for subsequent studies. If the material is not available, no label is needed.

Summaries for the past year: A list of the gene symbols approved for soybeans from March 1989 to April 1990 is given in Table 1. Previously approved genes that have been published recently are given in Table 2.

It has been brought to the attention of the committee that the gene symbols Pst/pst, (resistance/susceptibility to peanut stripe virus) are published but were not submitted for approval. These genes have been added to the list of unapproved gene symbols.

Anyone knowing of gene symbols which do not appear in the Gene Symbol Index of the Soybean Monograph (1987, pages 192-196), or in Tables 1 and 2 in the Soybean Genetics Newsletter 16:4-5, or in this issue, please advise the Soybean Genetics Committee accordingly.

Brian McBlain
Past Chairman

References

Chandlee, J.M. and L.O. Vodkin. 1989. Unstable genes affecting chloroplast development in soybean. Devel. Genet. 10:532-541.

Choi, S.M., S.K. Green, D.R. Lee and J.Y. Yoon. 1989. Incompletely dominant single recessive gene for peanut stripe virus in soybean line ACS 129. Euphytica 40:193-196.

Griffin, J.D. and R.G. Palmer. 1989. Genetic studies with two superoxide-dismutase loci in soybean. Crop Sci. 29:968-971.

Matthews, A., B.J. Carroll and P.M. Greshoff. 1989. A new non-nodulation gene in soybean. J. Hered. 80:357-360.

Nickell, C.D., P.M. Hanson, L.E. Gray, D.J. Thomas and D.B. Wilmot. 1990. Registration of soybean germplasm lines LN86-1595 and LN86-1947 resistant to brown stem rot. Crop Sci. 30:241.

Palmer, R.D., R.W. Groose, B.R. Hedges and R.S. Benevente. 1989. W4 mutable line in soybean. Devel. Genet. 10:542-551.

Palmer, R.G. and H. Skorupska. 1990. Registration of a male-sterile genetic stock (T259H) of soybean. Crop Sci. 30:241.

Polacco, J.C., A.K. Judd, J.K. Dybing and S.R. Cianzio. 1989. A new mutant class of soybean lacks urease in leaves but not in leaf-derived callus or in roots. Mol. Gen. Genetics 217 N2-3:257-262.

Rennie, B.D., M.M. Cramer and W.D. Beversdorf. 1989. Can. J. Plant Sci. 68:793-795.

Rennie, B.D. and J.W. Tanner. 1989. Comparison of fan alleles in C1640 and the lines A5, PI 123440 and PI 361088B. Soybean Genet. Newsl. 16:23-25.

Rhodes, P.R. and L.O. Vodkin. 1988. Organization of the Tgm family of transposable elements in soybean. Genetics 120-2:597-604.

Skorupska, H. and R.G. Palmer. 1989. Genetics and cytology of Ms6 male-sterile soybean. J. Hered. 80:304-310.

Tajagi, Y., M. Hossain, T. Yanagita, and S. Kusaba. 1989. High linolenic acid mutant in soybean induced by X-ray irradiation. Japan. J. Breed. 39:403-409.

Wilcox, J.R. and J.F. Cavins. 1990. Registration of C1726 and C1727 soybean germplasm with altered levels of palmitic acid. Crop Sci. 30-240.

Table 1. Soybean gene symbols and linkages approved March 1989-April 1990.

Date	Authors	Trait/Linkage	Gene/Linkage
July 1989	Hedges et al.	Linkage Group 11	F 24.7 Idh1 Idh1 26.9 Rj1
July 1989	Specht et al.	Linkage Group 18	Mpi-dt2
Aug. 1989	Honeycutt et al.	Variegated leaf	V2/v2
Sept. 1989	Rennie et al.	Phytophthora resistance	Rsp?(Neuzumisaya)
Feb. 1990	Keen and Buzzell	Pseudomonas resistance	Rpg2/rpg2, Rpg3/rpg3 and Rpg4/rpg4
Feb. 1990	Wilcox and Abney	Narrow rugose leaf	Lnr/lnr
Feb. 1990	Chen et al.	SMV resistance	RsvY, Rsvm', and Rsvk
April 1990	Fehr et al.	Low palmitic acid	fapx

Table 2. Annual list of recent publications with approved gene symbols.

Gene	Strain	Phenotype	Reference
fan	PI 123440 A5	Low linolenic acid	9, 10
fap1	C1726	Low palmitic acid	14
fap2	C1727	High palmitic acid	14
Ms6	T259H	Male sterility	7, 12
Rbs2	LN86-1595	Brown stem rot resistance	5
	LN86-1947	Brown stem rot resistance	
Sod2-a	Polysoy	Zone II & III bands	3
Sod2-b	Williams 82	Zone II & III bands	
w4-m	Asgrow X25AF	Mutable flower color	6

USDA NORTHERN SOYBEAN GERMPLASM REPORT

In 1989, a total of 17,944 seedlots were distributed from the USDA Northern Soybean Germplasm Collection in response to 325 requests from 31 states and 27 foreign countries. Seed orders were placed by 260 U.S. requestors for 14,123 seedlots, and by 65 foreign requestors for the remaining 3,821.

Of the approximately 8,600 Glycine max strains in the Urbana collection, 1,200 were grown in 4-row plots in 1989 for germplasm maintenance. After the 1989 harvest, 41 accessions were added to the collection and are now available for distribution. Twenty-six of the additions were from China, 5 from Yugoslavia, 3 each from Japan and the Asian Vegetable Research and Development Center, and the remainder from various sources. The second year of general evaluation was completed for 513 accessions at Urbana and 43 accessions at St. Paul, MN, with the assistance of J. H. Orf. Because of the severe drought in 1988, these tests will be repeated in 1990.

There were 60 new additions to the wild soybean collection in 1989, with all but two of these coming from N.I. Vavilov All-Union Institute of Plant Industry, Leningrad, USSR. The current inventory of the USDA Wild Soybean Germplasm Collection consists of 909 accessions.

During 1989, 85 soybean accessions were received at Urbana. Of these, 25 were received from the Institute of Crop Germplasm Resources, Beijing, PRC; 15 from the Jilin Academy of Agricultural Sciences, Jilin, PRC; 4 from the Academy of Agricultural and Foresty Science, Hebei, PRC; and 14 from a visiting delegation from Hebei Province, PRC, via Iowa State University. Another 22 accessions came from various sources in Japan, 4 from Taiwan and 1 from the Republic of Korea. New acquisitions were grown this past season in comparison tests with 51 strains already in the collection which had similar identification. Twenty-one duplications were identified and discarded. The remainder will be available for distribution in late 1990.

New soybean accessions to be planted in 1990 include 29 collected in Nepal on an IBPGR-sponsored exploration, 2 from the Republic of Korea and one each from Taiwan and the Federal Republic of Germany. Seven accessions of Glycine max and 44 accessions of Glycine soja, sent to us by the N.I. Vavilov All-Union Institute of Plant Industry, Leningrad, USSR, will be included in this year's planting, along with six wild soybean accessions from the People's Republic of China.

The current inventory of the USDA Perennial Glycine Germplasm Collection consists of 815 accessions representing 15 species. Representatives of three recently identified species, G. albicans, G. hirticaulis and G. lactovirens, were added in 1989. During the year, 335 accessions were received from CSIRO in Australia. Seed multiplication was conducted in the greenhouse during the summer. Of the total collection, 348 accessions are currently available for distribution. During 1989, 497 seedlots were sent in response to 22 requests from 6 states and 5 foreign countries. Seed multiplication and distribution is done in cooperation with T. Hymowitz, University of Illinois.

As of January 1, 1990, all seed requests will be processed through

the Germplasm Resources Information Network (GRIN). Integration of this system with a local database manager will allow us to produce summary reports of germplasm distribution more easily, to keep more accurate inventory information, and to provide more information to requestors about the material they receive. Collection users in Mexico, Canada, and the United States can now query the soybean germplasm database and place orders through the Germplasm Resources Information Network. For further information contact: The Database Manager, Building 001, Room 130, BARC-West, Beltsville, Maryland 20705 U.S.A. Telephone 301/344-1666.

In October 1989, Claudia T. Coble became assistant curator of the USDA Northern Soybean Germplasm Collection. She received a Master of Science degree in agronomy at Kansas State University while working with William T. Schapaugh,, Jr., as an agricultural technician. Before joining the USDA, she was employed by the Department of Agronomy at the University of Illinois.

New publications available for distribution from the USDA Northern Germplasm Collection in 1989 include the following:

Bernard, R.L., G.A. Juvik and R.L. Nelson. 1989. USDA Soybean Germplasm Collection Inventory. Vol. 2. International Agricultural Publications. INTSOY Series No. 31. (Origin information on strains between PI 150,000 and PI 500,000).

Juvik, G.A., R.L. Bernard, J.H. Orf, J.F. Cavins and D.J. Thomas. 1989. Evaluation of the USDA Soybean Germplasm Collection: Maturity Groups 000 to IV (PI 446,893 to PI 486,355). USDA Technical Bulletin No. 1760. (Includes 68 domestic cultivars released before 1988).

Juvik, G.A., R.L. Bernard, R. Chang and J.F. Cavins. 1989. Evaluation of the USDA Wild Soybean Germplasm Collection: Maturity Groups 000 to IV (PI 65,549 to PI 483,464). USDA Technical Bulletin No. 1761.

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USDA SOUTHERN SOYBEAN GERMPLASM COLLECTION REPORT

February, 1990

Maturity group	Total entries 1984	Total entries 1987	Total entries 1988	Total entries 1989
V	1549	1550	1813	1829
VI	482	487	787	797
VII	346	351	449	456
VIII	297	308	352	355
IX	131	149	149	153
X	154	165	165	165
	2959	3010	3715	3755

Eighteen new accessions were added to the GRIN system and to the Southern soybean collection.
In 1990, Group V, Line Numbers 701-1400 will be increased at Stoneville. Group IX, Line Numbers 1-153 will be increased at Gainesville, Florida.
Seed requests filled in 1989: 403 packets to 13 countries and 12,804 to 32 states. Total volume of packets for 1989 was 13,207.

C. J. Edwards, Jr.
E. E. Hartwig

SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (CAC) held its annual meeting 26 Feb. 1990, at the Soybean Breeders Workshop in St. Louis, MO. Eleven of the thirteen members were in attendance. Those elected to three-year terms were: H. Roger Boerma, University of Georgia, Athens, GA; John Thorne, the New Northrup King Co., Washington, IA; and Lawrence D. Young, USDA-ARS, Jackson, TN. Dr. Boerma and Dr. Thorne were at the meeting. Also in attendance were: Claudia J. Coble, Assistant Curator of the Soybean Germplasm Collection; Gary R. Ablett, Public Breeder, invited observer from Canada; Allan K. Stoner, of Germplasm Services Laboratory; and James R. Wilcox, USDA-ARS, invited participant.

The Soybean Germplasm CAC By-laws were published in the Soybean Genetics Newsletter 16:14-15, 1989. These By-laws were reviewed. Randall Nelson proposed that the second sentence of the By-laws be changed from "The curators of the northern and southern germplasm..." to "The curators and assistant curators of the northern and southern germplasm..." A motion was made and passed to make this change. This change is included in the By-laws published on page 13 of this issue.

Thomas Kilen read a letter from Robert R. Kalton, Vice-chairman of the Public Research Advisory Committee of the American Seed Trade Association. The letter expressed a concern over the small number (two) of seed industry members on the CAC. A discussion brought out the fact that in addition to the private breeder, North, and the private breeder, South, industry representatives are eligible to serve on five other positions on the CAC if their discipline matches those positions. Dr. Nelson suggested a list of people be generated who are interested in germplasm, its enhancement and use. James Orf suggested that curators could identify people who utilize germplasm and offer suggestions for determining potential soybean CAC members. Also, commercial breeders could provide names of people in disciplines other than breeding, such as biotechnology, plant physiology, or plant pathology and pass them on to the Chairman of the CAC.

Updates on both northern and southern portions of the USDA Soybean Germplasm Collection were given by Claudia Coble, Randall Nelson, and Edgar Hartwig. They reported that over 18,000 and 13,000 seed requests were filled from northern and southern germplasm collections, respectively. Dr. Nelson reported that the National Seed Storage Laboratory (NSSL) would like to store larger quantities of seed. Therefore, the northern germplasm maintenance plots will be harvested from a larger area, and will be stored in larger containers. Dr. Kilen reported that most of the southern germplasm collection has been evaluated for resistance to Races 3, 4 (14), and 5 of soybean cyst nematode. The remainder of the southern germplasm will be evaluated for resistance to soybean looper in 1990. Reports on the current germplasm collections are presented on pages 6-8.

Lavone Lambert read a letter from Michael E. Irwin, Entomologist, University of Illinois, indicating a need to eliminate the virus-infected seed in the germplasm collection. Dr. Nelson indicated the amount of virus could be reduced through insect control, but not entirely eliminated. He indicated that restrictions and standards for seed sent to other countries are stringent

and hard to meet. Dr. Lambert suggested a CAC subcommittee be established to study the extent of the problem and to suggest ways to control seed-transmitted viruses in the germplasm collection.

Dr. Nelson presented three curator needs outlined in the February 5 memorandum sent to each soybean CAC member. Most discussions centered on whether or not varieties having a Plant Variety Protection (PVP) certificate should be distributed to requestors or if all requests should be referred to the donor. PVP varieties are listed in the Germplasm Resources Information Network (GRIN) as unavailable until protection expires. Therefore, a request must be made to the originator. It was suggested that a subcommittee might be formed to determine a better way to handle seed requests of protected varieties. Dr. Nelson also issued a concern on germplasm evaluation and documentation on the GRIN system. He raised questions as to which publications are useful and what information needs to be included in these documents. It was suggested that this also may be best determined by a CAC subcommittee.

Alan Stoner presented an update on the GRIN system. All germplasm orders are processed through the GRIN system to better track germplasm use and provide more information to the user as well as allow more information to be added to the working collection. There are now over 500 users who have access to GRIN. Interested parties who want access to GRIN, including a user's manual, may call Jimmie D. Mowder, GRIN Database Manager, at 301/344-3318. GRIN is available to any scientist in the United States, Canada, and Mexico, plus the thirteen germplasm centers around the world. New data are being continuously added to the system. The CAC committee has the responsibility of determining what goes into GRIN.

Dr. Nelson reported that two germplasm requests had been made to the People's Republic of China, for 500 accessions each from Beijing and Jilin. None have been received. One problem is that seed requests have depleted their seed supplies. Henry Shands will visit China in March and will inquire about these requests.

An update on a collection of privately grown varieties was given by Dr. Nelson. Few applications have been received for submission of private varieties to the collection. Publicity will be continued with covering letters and applications to submit private varieties to the collection. Varieties that should be added to the collection should be widely grown, be quoted in journal publications, have a unique pedigree or used in the development of other varieties, and have some unique trait or traits.

High protein germplasm enhancement was discussed. James Wilcox sent out a questionnaire to public and private breeders to identify breeding material with high protein. He expressed a willingness to coordinate a test in 1990 of Northern soybean lines with high protein. Dr. Kilen gave committee members a review of breeding for high protein in public and private programs. Dr. Nelson presented information concerning high protein accessions in various maturity groups. He suggested planting these lines in hills at four locations to get more accurate data on protein stability across environments. Dr. Orf suggested that requests be made to release more adapted material with high protein and add it to the soybean germplasm collection for more useful breeding material.

The committee unanimously passed a resolution expressing appreciation to Calton Edwards, USDA-ARS, Stoneville, MS, for his significant contribution as manager of the collection at Stoneville for nearly as many years as the collection has existed.

Special thanks were extended to Kuell Hinson, USDA-ARS, Gainesville, FL (six years of service); Clark Jennings, Pioneer Hibred International, Waterloo, IA (six years of service); and Donald Schmitt, North Carolina State University, Raleigh, NC (three years of service) for their active participation on the CAC. Thomas Kilen and Clark Jennings were thanked for serving for five years as CAC Chairman and Vice-chairman, respectively.

Elections were held for the positions of chairman and vice-chairman. James Orf, University of Minnesota, and Grover Shannon, Delta and Pine Land Company, Scott, MS, were elected as chairman and vice-chairman, respectively. Committee members were asked to volunteer for the various CAC subcommittees: (1) Acquisition, (2) Operations, and (3) Evaluation.

There being no other business, the meeting was adjourned.

Thomas C. Kilen, Past Chairman
Soybean Germplasm Crop Advisory Committee

Following are the current members, addresses, phone numbers, areas of representation, and years of service:

Name	Address	Area of Representation	Years of service
T. Scott Abney 317/494-9859	USDA-ARS Dept. of Botany & Plant Path. Purdue Univ. W. Lafayette IN 47907	Plant Pathology	1986-89 1989-92
H. Roger Boerma 404/542-0927	Dept. of Agron. Univ. of Georgia Athens GA 30602	Public Breeding South	1990-93
Claudia J. Coble 217/244-4345	USDA-ARS Dept. of Agron. Univ. of Illinois 1102 S. Goodwin Urbana IL 61801	USDA Germplasm Collection	ex officio
Dennis B. Egli 606/257-7317	Dept. of Agron. Univ. of Kentucky Lexington KY 40546	Physiology	1989-92
Edgar E. Hartwig 601/686-9311	USDA-ARS Soybean Prod. Res. PO Box 196 Stoneville MS 38776	USDA Germplasm Collection	ex officio

Name	Address	Area of representation	Years of service
Thomas C. Kilen 601/686-9311	USDA-ARS Soybean Prod. Res. PO Box 196 Stoneville MS 38776	USDA Germplasm Collection	ex officio
Lavone Lambert 601/686-2311	USDA-ARS Southern Field Crop Insect Mgmt. Lab. PO Box 346 Stoneville MS 38776	Entomology	1988-91
Philip Miller 202/344-2725	USDA-ARS Beltsville Agric. Res. Center Bldg. 005, BARC-West Beltsville MD 20705	USDA Natl. Program staff	ex officio
Randall Nelson 217/244-4346	USDA-ARS Dept. of Agron Univ. of Illinois 1102 S. Goodwin Urbana IL 61801	USDA Germplasm Collection	ex officio
J. H. Orf 612/625-8275	Dept. of Agron. and Plant Genetics Univ. of Minnesota St. Paul MN 55108	Public Breeding North	1985-88 1988-91
Reid G. Palmer 515/294-7378	USDA-ARS G-301 Agronomy Iowa State Univeristy Ames IA 50011	Cytogenetics & Molecular Genetics	1985-88
J. Grover Shannon 601/742-3351	Delta & Pine Land Co., PO Box 157 Scott MS 38772	Private Breeding South	1986-89 1989-92
John Thorne 319/653-2181	The New Northrup King Co., PO Box 949 510 N. 12th Washington IA 52353	Private Breeding North	1990-93
Lawrence D. Young 901/425-4741	USDA-ARS West Tennessee Exp. Stn. Jackson MS 38301	Nematology	1990-93

SOYBEAN GERMPLASM CROP ADVISORY

COMMITTEE BY-LAWS

Membership

The Soybean Germplasm Crop Advisory Committee will consist of 14 members. The curators and assistant curators of the northern and southern portion of the collection, the research geneticists working with the germplasm collection at each location, and a representative from the National Program Staff will serve as ex officio members. All ex officio members will have full voting privileges and may hold committee offices.

The remaining nine committee members will be elected to the committee to represent various geographical and/or research areas as follows:

1. Private breeder, north
2. Private breeder, south
3. Public breeder, north
4. Public breeder, south
5. Pathologist or nematologist
6. Pathologist or nematologist
7. Entomologist
8. Physiologist or biochemist
9. Cytogeneticist or molecular geneticist

Terms of Office

Committee members will be elected to 3-year terms and may serve no more than 2 consecutive terms. After an absence of at least one year from the committee, a former two-term member is eligible for membership again. Terms will begin following the annual meeting held in conjunction with the Soybean Breeders' Workshop in late February.

Committee Officers

The committee shall have two elected officers, chairperson and vice-chairperson. Each officer will be elected to a one-year term at the committee's annual meeting to serve the following year and may serve no more than five terms out of any 8-year period.

The duties of the committee chairperson include coordinating annual elections, notifying members of meetings, chairing meetings, and other duties as necessary to fulfill the committee's responsibilities. The vice-chairperson shall record the proceedings of all meetings and assist the chairperson as requested.

Sub-committees

Standing sub-committees shall be established and approved by a majority vote of the committee. Ad hoc sub-committees shall be established as needed by the chairperson.

Elections

Each year three memebers will be elected in the following manner:

Year 1: Entomologist
Northern breeder, public
Cytogeneticist or molecular geneticist
Year 2: Southern breeder, private
Physiologist
Pathologist or nematologist (position A)

Year 3: Northern breeder, private
Southern breeder, public
Pathologist or nematologist (position B)

By November 1 of each year, the chairperson will send a request for nominations for each position for which the incumbent's term expires the following February. These requests will be sent to those whose discipline and geographical areas are the same as the qualifications for the open committee position. All nominations must be received by the chairperson by November 30. If more than two nominations are received for any position, the chairperson will send all nominations to the committee and each member may vote for two candidates for each position. The ballots from this primary election must be sent by December 7 and returned to the chairperson by January 7. A final ballot with the top two candidates for each position will be sent to the committee by January 10. These ballots must be returned to the chairperson by January 27. The chairperson will then notify the newly elected members so that they may attend the annual meeting in late February. The newly elected members will officially begin their terms after that meeting but will be invited to attend as observers.

Rule Changes

These rules may be amended by a majority vote (8) of the committee members.

CANADA

RESEARCH NOTES

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and

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1) Linkage Tests with L1

Buzzell and Palmer (1989) reported a loose linkage of E3 with Rmd (43.6 ± 3.1) and that E3 was probably linked with dt1 which is in Linkage Group 5 (Weiss, 1970).

E3 and Rmd were tested for linkage with L1 using the cross of Norsoy (E3 Rmd l1) x T93A e3 rmd L1) (Table 1). E3 is not closely linked to L1 which Weiss (1970) reported to be linked to dt1 (39.4 ± 1.8). Thus E3 must occur on the other side of dt from L1. The segregation of E3e3 with Dt1dt1 is being tested in another cross to determine how close E3 is linked with Dt1.

Rmd is not linked to L1 (Table 1) and is unlikely to be linked with Dt1 since Rmd and E3 are loosely linked. Other gene markers are needed to determine with certainty as to whether Rmd is in the same linkage group as E3.

Table 1. F2 linkage tests for three pairs of alleles in the cross of Norsoy (E3 Rmd l1) x T93A (e3 rmd L1)*.

Alleles	Allele combinations† a	b	c	d	N	R ± SE ¶	Phase^
E3e3 Rmd rmd	127	52	32	16	227	47.8 ± 4.8	C
E3e3 L1 l1	135	44	37	11	227	48.7 ± 5.1	R
Rmd rmd L1 l1	117	42	55	13	227	44.1 ± 5.2	R

* Pod color of F2 plants was recorded and confirmed with F3 rows. Powdery mildew reaction and flowering response was determined on about 10 F3 plants for each F2 in a greenhouse with powdery mildew present and daylength extended to 20h with cool white fluorescent light.

† a=XY, b=Xy, c=xY, and d=xy for the three sets of allele pairs in the order of Xx and Yy.

¶ Recombination units and standard errors were obtained following Immer and Henderson (1943).

^ C = coupling; R = repulsion.

References

Buzzell, R.I. and R.G. Palmer. 1989. Rmd and E3 linkage. Soybean Genet. Newsl. 16:29-30.

Immer, F.R. and M.T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Weiss, M.G. 1970. Genetic linkage in soybeans. Linkage groups V and VI. Crop Sci. 10:469-470.

R. I. Buzzell
B. D. Rennie

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1) Linkage assays.

Genetic linkage assays were performed on two F2 populations segregating for a total of 28 pairs of loci. The two crosses used were Norsoy x T31 and PI 361088B x PI 423935. The genetic traits indicated by the allele symbols in Tables 1, 2, and 3 are described by Palmer and Kilen (1987).

Twelve of the 28 linkage assays have not been reported in the literature previously. In all of these assays the loci pairs were found to be segregating independently. In the 16 linkage assays that have been reported previously, no evidence of linkage was found except in the combinations Idh2 - Fan, and P2 - Ln. The Idh2 and Fan loci have been shown to be in Linkage Group 17, linked at a distance of 26.2 ± 1.2 units (Rennie and Tanner, 1989), while the P2 and Ln loci have been shown to be in Linkage Group 4, linked at a distance of 26.4 ± 1.4 units (Weiss, 1970). The linkage estimates for these two loci pairs in the current study are in agreement with the published values.

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Table 1. F2 segregation data for alleles from pairs of loci in the progeny of the crosses Norsoy x T31 and PI 361088B x PI 423935, segregating in 4:2:2:2:2:1:1:1:1 ratios.

Cross*	Locus pair	Ab+aB aB Ab	Ab aB	Ab ab	AB Ab	aB ab	AB AB	aB aB	Ab Ab	ab ab	N	X^2	P
1	Aco2-Idh2	47	21	24	13	15	11	8	7	12	158	7.65	<0.30
2	Aco4-Fan	17	9	11	9	10	8	6	5	9	84	5.44	<0.70
2 ^	Aco4-I	21	10	8	12	13	7	2	3	10	86	9.02	<0.30
2	Aco4-Idh2	25	13	2	11	12	7	7	4	6	86	9.59	<0.20
2	Aco4-Pgm1	19	9	11	8	17	11	1	3	7	86	15.91	<0.05
2	Idh2-Fan	38	14	15	13	9	6	14	22	9	133	32.66	<0.001
2 ^	Idh2-I	38	16	15	20	12	12	3	9	10	135	8.12	<0.30
2	Idh2-Pgm1	38	16	15	22	12	10	6	9	7	135	5.03	<0.70
2	Pgm1-Fan	37	16	19	11	12	8	10	13	8	134	6.92	<0.50
2 ^	Pgm1-I	46	11	16	11	14	10	8	11	9	136	10.13	<0.20
2 ^	Fan-I	37	10	18	18	16	9	10	9	14	141	6.77	<0.50

*Cross 1 = Norsoy x T31, cross 2 = PI 361088B x PI 423935.
^ Indicates that this linkage assay has not been reported previously.

Table 2. F2 segregation data for alleles from pairs of loci in the progeny of the crosses Norsoy x T31 and PI 361088B x PI 423935, segregating in 6:3:3:2:1:1 ratios.

Cross*	Locus pair	Aa B_	AA B_	aa B_	Aa bb	AA bb	aa bb	N	X^2	P
1 ^	Aco2-I	74	23	28	18	9	7	159	6.28	<0.20
1 ^	Aco2-Ln	70	24	27	22	8	8	159	4.20	<0.50
1 ^	Aco2-P2	78	23	29	13	9	7	159	10.58	<0.05
1	Aco2-T	76	25	24	16	6	12	159	9.16	<0.10
1	Idh2-I	71	32	38	12	15	8	176	7.99	<0.10
1 ^	Idh2-Ln	59	31	38	24	9	10	171	2.18	<0.70
2	Idh2-Ln	53	30	23	16	11	2	135	6.93	<0.20
1 ^	Idh2-P2	71	35	37	13	6	11	173	7.01	<0.20
1	Idh2-T	66	30	36	17	10	12	171	1.75	<0.70
2	Aco4-Ln	30	18	18	9	4	7	86	1.72	<0.70
2 ^	Pgm1-Ln	59	22	24	14	10	7	136	2.88	<0.70
2 ^	Fan-Ln	51	30	30	15	6	11	143	2.80	<0.70
2 ^	I-Ln	55	23	33	17	8	7	143	2.57	<0.70

*Cross 1 = Norsoy x T31, cross 2 = PI 361088B x PI 423935.
^ Indicates that this linkage assay has not been reported previously.

Table 3. F2 segregation data for alleles from pairs of loci in the progeny of the crosses Norsoy x T31 and PI 361088B x PI 423935, segregating in 9:3:3:1, or 9:3:4 ratios.

Cross*	Locus pair	A_ B_	A_ bb	aa B_	aa bb	N	R	SE
1	I-Ln	103	28	38	7	176	44.5	5.9
1	I-P2	110	32	29	5	176	>55	
1	Ln-P2	108	39	26	3	176	34.5	6.5
1	Ln-T	101	34	31	10	176	49.5	5.7
1	P2-T	106	28	37	5	176	>55	
							X^2	P
1	I-T	106	35	35	--^	176	2.45	<0.20

* Cross 1 = Norsoy x T31, cross 2 = PI 361088B x PI 423935.
^ The expected ratio is 9:3:4 due to the interaction of alleles at the I and T loci.

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1) Genetic implication of soybean resistance to Cercospora sojina race 1.

Abstract: Four crosses were made between the pure breeding varieties 81-732 and 'Ozzie' which are resistant to race 1 of Cercospora sojina, and susceptible pure breeding varieties 'Suinong 4' and 'Amsoy'. Preliminary evidence from inoculations of four F2 populations indicated that resistance to C. sojina race 1 was conditioned by a single dominant gene. In order to confirm the hypothesis of a single dominant gene controlling the inheritance of resistance to C. sojina race 1, the study on segregation of 198 F3 lines of cross 81-732 x Suinong 4 was made. Frequency distribution curve for gene analyses to the F3 generation indicated that the observed curve fitted well with expected distribution curve for one completely dominant resistant gene with no existence of minor genes or complementary genes.

Introduction: Frogeye leaf spot, caused in soybean by Cercospora sojina Hara, was first reported in 1915 in Japan and is known worldwide. It is most common in warmer regions during warm, humid weather. Yields from susceptible cultivars may be reduced 12-15%, even up to 30%. Quality of infected seed is lowered both by discoloration and reduced germination. In general, it reduced seed germination, seedling vigor, protein content (about 1.2%), and oil content (2.9%). The disease has caused major economic problems in soybean production in China. So far, the disease has been studied in three respects: epidemic study of Cercospora sojina Hara, inheritance of resistance to the disease in soybean, and breeding resistant soybean cultivars.

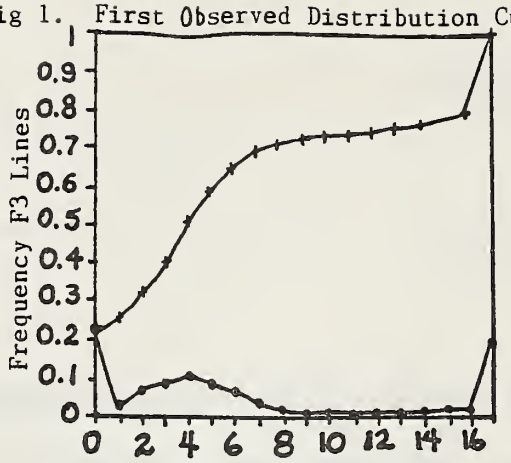
In the United States, 11 physiological races of the fungus have been identified. Resistance to race 1 and race 2 of the US is conditioned by the genes Rcs1 and Rcs2, respectively. Rcs3 gene conditions resistance to races 2 and 5 in the cultivar 'Davis'. In China, Heilongjiang Agricultural Institute has identified 11 physiological races and detected the distribution of these races in Heilongjiang Province; the dominant races are races 1, 7, and 10. The study of comparison between American Cercospora sojina races and the Chinese Cercospora sojina races has not been carried out.

The objective of the present study was to find and prove the inheritance of resistance to Chinese C. sojina race 1.

Materials and methods: Pure breeding varieties resistant to race 1, 81-732 and Ozzie, and susceptible pure breeding varieties Suinong 4 and 73-876, were used as parents in the following crosses: 81-732 x Suinong 4, 81-732 x 73-826, Ozzie x Suinong 4, and Ozzie x 73-726.

In 1989 all F1 plants were grown in the breeding nursery of Northeast

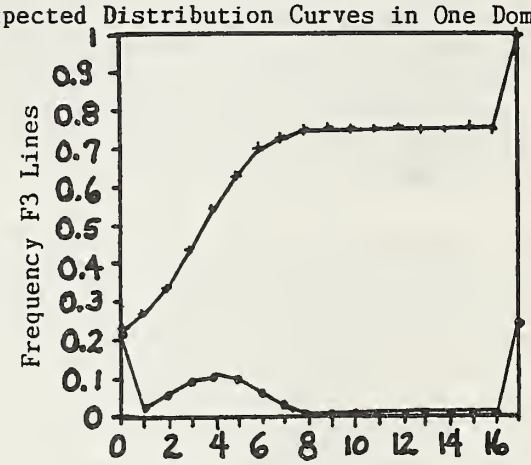
Fig 1. First Observed Distribution Curves



The number of susceptible plants

• Fre. Dis. Curve + Cum. Dis. Curve

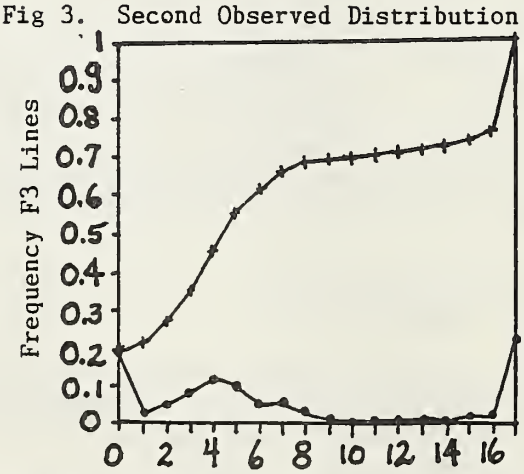
Fig 2. Expected Distribution Curves in One Dominant Resistant Gene



The number of susceptible plants

• Fre. Dis. Curve + Cum. Dis. Curve

Fig 3. Second Observed Distribution Curves



The number of susceptible and intermediate plants

• Fre. Dis. Curve + Cum. Dis. Curve

Agricultural College in Harbin City. In the same year, a part of the F1 seeds were planted in plastic troughs in the greenhouse. The remainder of the seeds were planted in the breeding nursery in 1988. One hundred ninety eight different F2 plants of cross 81-732 x Suinong 4 were sorted out from the breeding nursery at random. Their seeds for F3 were planted individually in different F3 rows of plastic troughs in the greenhouse in 1989. Parent varieties were included in each test.

All plants growing in the greenhouse were inoculated by spraying with mycelial-spore suspension at the two trifoliolate leaf stage. Race 1 came from Hejiang Agricultural Institute, and culture of race 1 was maintained in PDA medium in test tubes. The mycelia in the PDA medium were incubated on sorghum medium for 14-16 days at 26-27 C and then the sorghum medium was incubated under 27-29 C and high humidity for 72 h. A large amount of spores can be obtained. The medium with spores was macerated in pure water with 3% sugar. After filtering, the final mycelial-spore suspension contained 3-4 conidia under 100x microscope field. After inoculation, a piece of clear plastic membrane was placed over the plants for 72 hr to maintain high relative humidity.

Rating was undertaken 18 days after inoculation. Rating standard was based on size of lesion and numbers of lesions on the inoculated leaves. Size of lesion was divided into four rates: B (large spreading lesion), M (large lesion), N (small lesion), S (flake). And number of lesions on the trifoliolate leaf was divided into five rates: 4, 3, 2, 1, and 0. The four rates of size of lesion, B, M, N, and S, were given weighting coefficients 4, 3, 2, and 1, respectively, so level of the disease had 10 rates, finally (Table 1). Plants that showed 4, 3, 2, 1, and 0 rates were classified as resistant; plants that showed 16, 12, and 9 rates were classified as susceptible; plants that showed 8 and 6 were intermediate.

Results and discussion: All F1 plants of the four crosses in the breeding nursery showed higher resistance levels in 1987, when the disease was very serious. Plants that showed more serious than rate 9 were not found, so all F1 plants were considered to be resistant.

The F2 populations from the 81-732 x Suinong 4 were counted; 81-732 x Amsoy, Ozzie x Amsoy crosses segregated in a ratio of 3 resistant : 1 susceptible. All the Chi-square values for tests for goodness of fit were acceptable, with Chi-square probability values greater than 0.15 (Table 2). In F2 segregation, intermediate plants were added into resistant plants.

These results indicate that a single dominant gene conditions the inheritance of resistance to C. sojina race 1. In order to confirm this hypothesis, the number of resistant, susceptible and intermediate plants in each F3 line of cross 81-732 x Suinong 4 were counted. According to the number of susceptible plants in each F3 line, F3 lines can be classified into 18 different reacting types. Grouping the F3 lines with the same reacting type, then frequency of each reacting type of F3 lines was obtained (Table 3). The first observed frequency distribution curve was drawn on the number of susceptible plants, and first cumulative distribution was obtained by adding the frequencies from left (Fig. 1). By comparison with the expected binomial distribution curves (Fig. 2), the two observed curves fit well with the expected distribution of one single dominant resistance gene. Test of

significance of difference by Holmogorov-Smirnov test between the two cumulative distributions indicated that there is nonsignificance. The approximation of maximum of difference between the two cumulative distributions is 0.054, smaller than 0.075 (n=198; a=0.10).

But the above first cumulative distribution curve was drawn on the number of susceptible plants. Under the case that the intermediate response plants were classified together with the resistant plants. Therefore, although the observed distribution curve fitted the expected distribution in a monogenic hybrid with one dominant gene, this does not always mean a participation of one completely dominant gene. In order to clarify the completeness of dominance, the intermediate plants were grouped into the susceptible group. Then the F3 lines showed 18 new different reacting types, according to the number of susceptible plants, which contained intermediate plants. Each new reacting type, frequencies of each reacting type in group of F3 lines, and cumulative frequencies are shown in Table 4.

The second observed frequency curve and second cumulative distribution curve are shown in Figure 3. The result of test of significant difference between the first and the second observed cumulative frequency distribution showed that there was no significant difference between the first and the second. The approximation of maximum of difference between the two cumulative distributions is 0.060, smaller than 0.075 (n=198; a=0.10). This indicates that the single gene is completely dominant.

Above results indicate satisfactorily that the inheritance of resistance to Chinese C. sojina race 1 is conditioned by a single completely dominant gene, with no minor gene or complementary gene.

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Table 1. Standard of frogeye leaf spot.

No. of lesions & rate \ Size of lesion and coe weighting	S	N	M	B
	1	2	3	4
0	0	0	0	0
<4	1	1	2	3	4
4-7	2	2	4	6	8
8-9	3	3	6	9	12
>9	4	4	8	12	16

Table 2. Segregation for reaction to Chinese Cercospora sojina race 1 in the F3 population.

Cross	Resistant	Susceptible	Total	Chi-square probability
81-732 x Suinong 4	117	37	154	0.7 -0.8
81-732 x Amsoy	125	37	162	0.5 -0.6
Ozzie x Suinong 4	69	26	95	0.5 -0.6
Ozzie x Amsoy	119	31	150	0.2 -0.15

Table 3. Reacting type and frequency of F3 lines (First).

Reacting type distribution				
No. of R+I	No. of S	Number of lines	Frequency	Cumulative
17	0	47	0.237	0.237
16	1	4	0.020	0.257
15	2	13	0.066	0.323
14	3	17	0.086	0.409
13	4	21	0.106	0.515
12	5	16	0.081	0.596
11	6	13	0.066	0.662
10	7	7	0.035	0.697
9	8	3	0.015	0.712
8	9	2	0.010	0.723
7	10	2	0.010	0.733
6	11	1	0.005	0.738
5	12	1	0.005	0.743
4	13	2	0.010	0.753
3	14	3	0.015	0.768
2	15	4	0.020	0.788
1	16	3	0.015	0.804
0	17	39	0.197	1.000

R = resistant plant; I = intermediate plant; S = susceptible plant.

Table 4. Reacting type and frequency of F3 lines (Second)

Reacting type distribution				
No. of R	No. of S + I	Number of lines	Frequency	Cumulative
17	0	38	0.192	0.192
16	1	5	0.025	0.217
15	2	10	0.051	0.268
14	3	16	0.081	0.349
13	4	23	0.116	0.465
12	5	20	0.101	0.566
11	6	10	0.051	0.617
10	7	10	0.051	0.668
9	8	5	0.015	0.693
8	9	2	0.010	0.703
7	10	0	0.000	0.703
6	11	2	0.010	0.713
5	12	1	0.005	0.718
4	13	2	0.010	0.728
3	14	1	0.005	0.733
2	15	4	0.020	0.753
1	16	4	0.020	0.773
0	17	45	0.277	1.000

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1) Electrophoretic analysis of seed storage protein of wild soybean (G. soja) in China.

Protein content of soybean (G. max) seeds is about 40%, and that of the wild soybean (G. soja, known as the inbreeding ancestor) is higher, some genotypes more than 50% higher (Xu Bao et al., 1984). Soybean is well known and much utilized throughout the world as an oil and protein seed. Improvement of the quality of soybean protein is needed because of the limit of sulfur-containing amino acid content, correlated with the composition of storage protein. The content of sulfur-containing amino acids of the 11S fraction was higher than that of the 7S fraction. There were many investigations carried out about the storage protein of soybean seed in recent years. There have also been some important discoveries. Kitamura et al. (1981) found that the cultivar 'Keburi' lacked the a' subunit. Then Beachy et al. (1983) demonstrated this genotype lacked the structural gene of the a' subunit. Staswick et al. (1983) found that the cultivar 'Reiden' lacked the $A_5A_4A_3$ subunits. Hu Zhiang et al. (1986) found a fast-a' subunit variant genotype.

There have been a few studies on storage protein of wild soybean. The purpose of this study was to find some variant genotypes of storage protein from our germplasm, by using SDS-PAGE.

Materials and methods: Two hundred thirty eight wild soybeans, from 24 provinces, 10 semiwild and 10 cultivated soybean germplasms were used in this experiment.

Storage protein in soybean seeds was extracted following the method described by Hill et al. (1974), and the SDS polyacrylamide electrophoresis was performed by using the method of Sun et al. (1975). The Japanese CS-930 Scanner was used in gel scanning.

Results and discussion: 1. Three fast-a' subunit (Fig. 1-c,d) and one double-a' subunit (Fig. 2) variant genotypes were found in wild soybean. The double-a' subunit genotype was first found at present. All these variant genotypes came from the region of about 35 N latitude. There were no variants found in 20 semiwild and cultivated soybeans.

2. In order to know the difference of different subunits among different species, the electrophoretic plates of 10 wild, 10 semiwild, and 10 cultivated soybean genotypes were scanned, using the CS-930 Scanner. The results (Table 1) show that the order of relative content of different subunits for all species was S>b>a>a' and B subunits, and the ratio of 11S/7S of semiwild was higher than that of wild and cultivated ones.

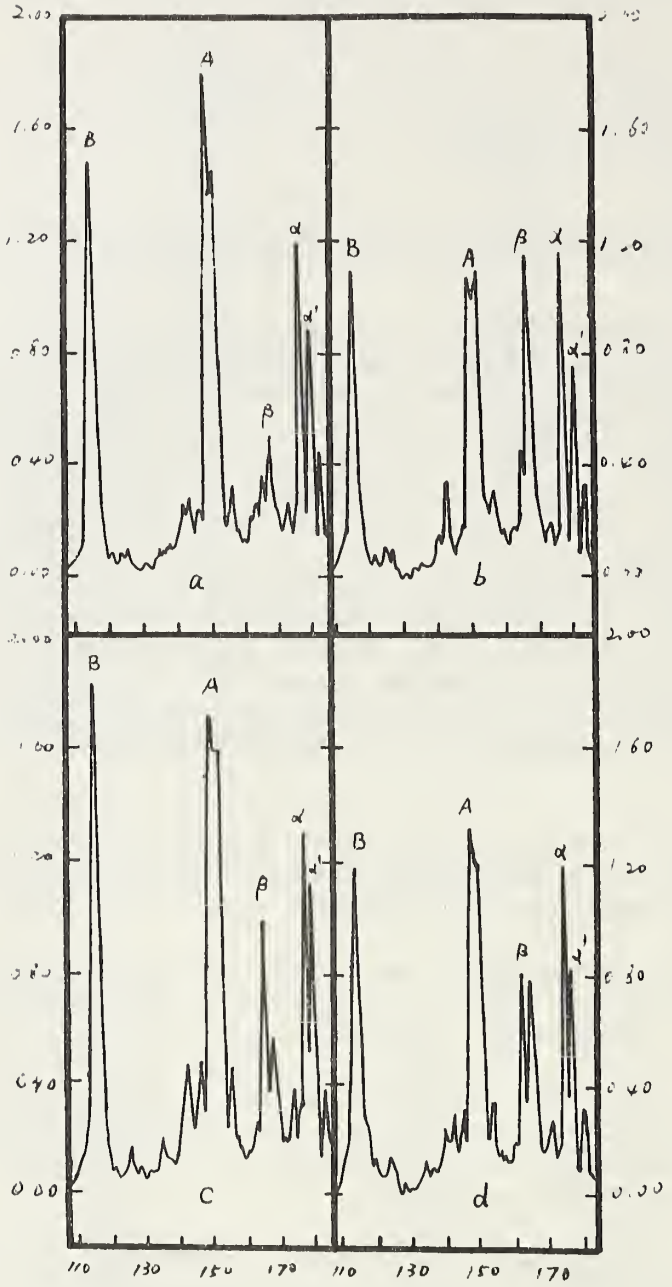

Figure 1. Scanning profile of storage protein in soybean seeds.

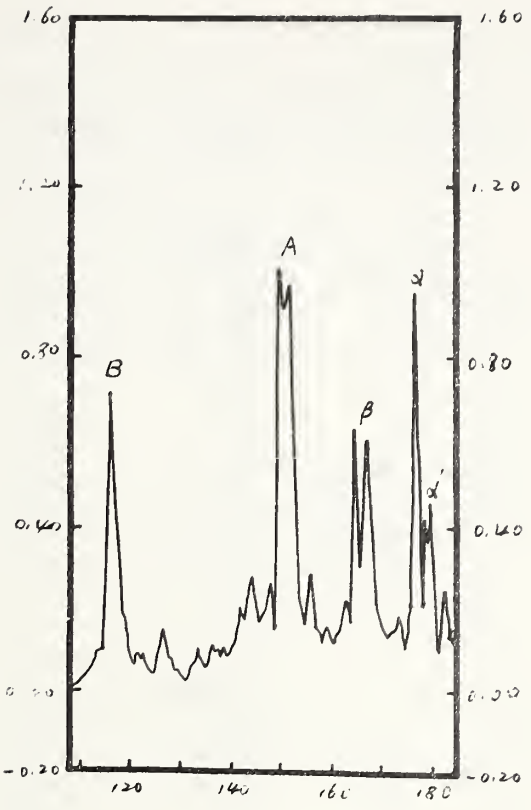

Figure 2. Scanning profile of double-a' subunit variant genotype.

There were many studies on soybean storage protein, but the double-a' subunit was the first found at present. It was seen that there may be more variant genotypes in Chinese soybean germplasm. The discovery of double-a' and fast-a' subunits is important to the study of gene structure, gene expression, gene replication and qualitative breeding.

It was found from this study that the level of the 11S fraction of semiwild soybean was higher than that of other species in the subgenus Soja. We also found a semiwild genotype characterized by a high level of the 11S fraction (the ratio of 11S/7S was more than 4) (in press). Kitamura and Kaizuma (1981) found that the Mo-Shi-Dou Gong 503 is characterized by a low level of both a' and B subunits of 7S globulin. All of these genotypes were found in semiwild germplasm. Therefore, the semiwild soybean should be emphasized in soybean qualitative breeding. It may be an important genetic resource that may contribute to the improvement of the protein composition in soybean seeds.

Table 1. Relative content of different subunits of storage protein in different soybean seeds.

Name of samples	Subunit a'	a	B	A	B	11S/7S*
			G. soja			
WO1	5.3	8.03	5.9	32.8	21.2	2.77
WO2	5.3	9.1	17.1	26.6	18.9	1.44
WO3	6.4	6.8	9.9	26.4	21.7	2.08
WO4	5.2	8.6	14.1	27.9	20.5	1.73
WO5	5.2	10.3	13.2	29.1	15.4	1.55
WO6	2.4	14.7	9.1	22.4	21.6	1.68
WO7	7.3	9.2	4.5	26.7	25.8	2.50
WO8	6.5	11.8	6.3	29.8	18.8	1.98
WO9	5.4	8.8	3.6	29.6	16.8	2.70
W10	6.3	9.7	5.5	30.3	14.6	2.09
$\bar{X}$	5.5	9.7	8.9	28.2	19.5	2.05
S	1.3	2.1	4.3	2.7	3.2	0.45
			G. gracilis			
SW01	6.9	8.5	6.0	22.1	25.6	2.55
SW02	6.9	7.9	5.6	31.1	25.2	2.76
SW03	5.5	13.1	9.9	26.6	24.5	1.79
SW04	8.9	8.7	8.2	32.6	22.0	2.12
SW05	8.0	7.6	8.4	21.2	18.7	1.66
SW06	8.1	10.6	8.1	26.4	22.8	1.82
SW07	8.4	8.8	4.8	34.8	27.9	2.85
SW08	10.1	10.1	5.8	28.8	26.0	2.11
SW09	9.1	8.0	5.6	29.3	24.8	2.38
SW10	6.4	8.7	4.1	25.5	26.1	2.69
$\bar{X}$	7.9	9.2	6.7	27.8	24.4	2.27
S	1.4	1.7	1.9	4.4	2.6	0.43
			G. max			
C01	11.8	7.1	7.6	32.1	21.5	-
C02	10.0	12.2	7.6	24.1	18.1	1.42
C03	8.0	10.8	13.4	24.0	16.6	1.26
C04	9.2	7.8	8.9	21.5	18.6	1.55
C05	10.3	11.7	6.2	29.3	21.5	1.80
C06	6.5	12.3	5.6	25.0	25.0	2.05
C07	9.5	14.7	9.9	22.5	18.6	1.21
C08	9.1	9.2	8.8	25.9	17.8	1.61
C09	7.9	11.7	6.0	31.6	18.0	1.94
C10	7.6	12.3	9.7	32.8	21.3	1.83
$\bar{X}$	9.0	11.0	8.4	26.9	19.7	1.63
S	1.5	2.3	2.3	4.2	2.5	0.30

* = $11S/7S=(A + B)/(\alpha + \alpha + \beta)$

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1) Application studies in the field on screening for cold-tolerance in the laboratory in soybeans.

Our experiment of 1987 and 1988 indicated that the germination percentages from different cultivars (strains) at a temperature of 6 C varied greatly, ranging from 0 to 100%, and that soybean cultivars with very small and kidney-shaped seeds, black seed coats, dark brown hila, and non-lustrous seed coats showed high tolerance to cold (Li et al., 1989a). When seeds treated at 6 C for 16 days were planted in the field, they had the ability to emerge, blossom and set pods (Li et al., 1989c) This screening test of cold-tolerance during germination at 6 C in chambers was combined with application studies during emergence in the field in early spring this year (1989) for confirming the reliability of the screening at low temperature in the laboratory. In the meantime, the emergence of soybeans in the field was affected by meteorological conditions and adaptability of soybean cultivars to the conditions of early spring were studied in this combined experiment.

Materials and methods: Screening for tolerance to cold during germination--A total of 130 cultivars from the northeast of China were tested again in 1989, which was similar to our preceding research (Li et al., 1989a, 1989b). After the test of germination at 6 C, three types of cold tolerance of soybean with three cultivars each type were chosen for the next experiment (Table 1).

Emergence experiment in early spring--A split-plot design was used with three planting dates, nine cultivars, and three replications in Beijing in early spring in 1989. The planting dates were 9 March, 29 March, and 18 April, once each 20 days. Some meteorological factors were recorded every day and others were provided by the Meteorological Research Laboratory of CAAS. Emergence in the field was investigated every day until the seeds in the field no longer appeared from the soil.

Statistical methods of cold-tolerance criteria and biological limited temperature--The germination index (GI), the germination percentage (GP), the emergence index (EI), the emergence potential (EP), the final emergence percentage (FEP), the initial emerging date (IED), and the biological limited temperature (BLT) were calculated according to the following formulae:

$$GI = \sum \frac{\text{No. of seeds germinated on day 1}}{\text{No. of days from treating at 6 C to that day 1}}$$

$$GP = \frac{\text{No. of seeds germinated at 6 C for 14 days}}{\text{Total of sowing seeds}} \times 100$$

$$EI = \sum \frac{\text{No. of seeds emerged on day 1}}{\text{No. of days from sowing to that day 1}}$$

$$EP = \frac{\text{No. of seeds emerged during top emerging stage}}{\text{Total of sowing seeds}} \times 100$$

$$FEP = \frac{\text{No. of seeds emerged from beginning to the end}}{\text{Total of sowing seeds}} \times 100$$

IED = No. of days when the first seed emerged from the soil and its cotyledons spread from sowing.

$$BLT = \frac{NaTa - NbTb}{Na-Nb}$$

Na, Nb are, separately, the number of days from sowing to the top emerging day in the a and b planting dates. Ta and Tb are, separately, the mean of air temperature each day from sowing to the top emerging day in the a and b planting dates.

Results: I. Screening of cold-tolerance during germination in soybeans--Thirty one (23.8%) cultivars were resistant to cold; 58 (44.6%) cultivars were mid-resistant to cold; and 41 (31.5%) cultivars were susceptible to cold during germination in chambers in 1989. Three types of cold tolerance with three cultivars each type (Table 1) were chosen randomly from them for the emergence experiment in early spring in the field.

II. The top emerging date, the biological limited temperature and the effective accumulation of air temperatures for soybeans emerging in the field--The earlier the sowing date in the spring, the longer time was needed for emergence in the field. Thirty days from sowing on 9 March, 16 days from sowing on 29 March, and 12 days from sowing on 18 April were needed, separately, for seeds to emerge up to the highest mean number of emergence on a day (Table 2). Those days were considered as the top emerging dates in their planting dates. According to the formula above, with the number of days needed in the top emerging stage and the mean of air temperature on a day recorded every day in the top emerging date in each planting date, 7.1 C was calculated as the biological limited temperature and 13 C, which was the effective accumulation of air temperature for soybean emerging up to the top emerging day from sowing, was discovered in the first time at the base of biological limited temperature.

III. Emergence and adaptability to meteorological conditions in early spring in soybean--The main treatments (sowing dates) and the sub-treatments (cultivars) of the emergence index, the initial emerging date, the emergence potential and final emergence percentage and the reciprocal effects of the main treatments and sub-treatments of the emergence index and the final emergence percentage were up to 1% significant level in the F test (Table 3).

Differences among planting dates--The normal sowing date for soybean in Beijing is 25 April. Earlier planting dates caused great delay in emergence in the field, and decreased significantly the emergence index, the emergence

potential, and the final emergence percentage (Table 4).

Variations among cultivars--B3, B9, and B6 cultivars, with black seed coats, emerged not only earliest, but also fastest and their final emergence percentages were highest in all cultivars sown in the field (Table 5), which indicates that soybean cultivars with black seed coats show the strongest resistance to bad meteorological conditions in early spring. On the other hand, B8 cultivar, with green seed coat, and B4 and B7 cultivars, with yellow seed coats, were susceptible to meteorological conditions in early spring. B1 cultivar, with yellow seed coat, and B5 and B2 cultivars, with green seed coats, exhibited mid-adaptability to early spring conditions.

Differences among cultivars within a planting date--Emergence expressions of B3, B9, and B6 soybean cultivars, with black seed coats, in every planting date, were the best or better (Table 6), which shows that B3, B9, and B6 cultivars had the strongest, or stronger, ability to resist low temperatures of early spring. Oppositely, B8, B4, and B7 cultivars had less ability to emerge in the field in each planting date in early spring, indicating that they were susceptible to early spring conditions, especially to cold injury. B1, B2, and B5 cultivars were located in the middle in every sowing date, stating clearly that they had a mid-resistance to bad environmental conditions in early spring.

IV. Relationship between emergence of soybean and meteorological factors--Total of mean number of emergences of soybean from initial emerging day to some day (Table 2) correlated positive and markedly with the means of temperatures on 5 cm, 10 cm, 15 cm, and 20 cm layers in the soil on a day up to 5% or 1% significance level, which was consistent among three sowing dates (Table 7), and indicated that the higher the temperature in the soil, the greater number of seeds emerged in the field. Other meteorological factors related to the total mean number of seeds emerging, correlated not as closely as did the temperature of the soil, demonstrating that the temperature of the soil was the key factor in inhibiting emergence of soybeans in the field in early spring. Delay of emergence from soil and decrease of number and percentage of emergence in earlier planting dates were due to lower temperatures of soil and air in early spring.

Discussion: Soybean cultivars B1-B9 were classified into three types of cold-tolerance, according to significant difference in germination index at 6 C in chambers (Table 1), which was also confirmed by the results of germination percentage at 6 C for 14 days (Table 8). The analysis above has shown that B3, B9, and B6 cultivars, with black seed coats, showed the strongest resistance to low temperatures of early spring. B1, B2, and B5 cultivars showed stronger resistance to the early spring conditions, and B8, B4, and B7 cultivars were susceptible. Thus it can be seen that when B3, B1, and B2 cultivars, which exhibited the highest resistance to cold in the laboratory, were used for emergence tests in the field, they showed the strongest or stronger resistance to cool injury in early spring in the field, especially B3, which was highly tolerant to cold during germination in laboratory and emergence in the field, proving that screening for cold-tolerance of soybeans in the laboratory was effective and usable.

Soybean cultivars with black seed coats (such as B3 and B9) showed the strongest resistance to low temperature conditions in early spring, which was

consistent with our preceding reports (Li et al., 1989a, 1989b) that soybean cultivars with black seed coats exhibited high resistance to cold during germination. Adaptability of soybean cultivars with yellow and green seed coats to meteorological conditions in early spring were worse, compared with the cultivars with black seed coats.

The correlation coefficient between the germination index in the laboratory and the emergence index in the field was higher, although it was not up to the 5% significance level, which was similar in correlation between germination percentage in the laboratory and final emergence percentage in the field (Table 8). Thus, we have shown that screening for tolerance to cold in soybean during germination in the laboratory was effective and reliable. Generally, soybean cultivars that show resistance to cold during germination in laboratory also show resistance or mid-resistance to cool injury during emergence in the field.

References:

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Table 1. Difference and classification of germination index of soybean at 6 C.

Cultivars	Seed coat color	Germination index	Significant at 1% level	Type of tolerance to cold
B3	Black	1.899	A	R
B1	Yellow	1.810	A	R
B2	Green	1.610	A	R
B5	Green	1.263	AB	MR
B6	Black	1.166	AB	NR
B4	Yellow	1.097	AB	MR
B9	Black	0.494	B	S
B8	Green	0.394	B	S
B7	Yellow	9.269	B	S

Table 2. Statistics of emergence of soybean seedlings on different planting dates.

On 9 March

Days	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Total of mean no. emergences on days	3.33	10.67	26.67	42.00	55.67	78.33	91.00	104.33	115.00	123.67	129.33	134.33	137.00	138.33	141.33	145.67	147.33	150.67	152.67	152.67
Mean no. of emergences on a day	3.33	7.34	16.00	15.33	13.67	22.66	12.67	13.33	10.67	8.67	5.66	5.00	2.67	1.33	3.00	4.34	1.66	3.34	2.00	0.00

On 29 March

Days	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Total of mean no. emergences on days	0.67	5.33	21.00	69.67	93.33	110.33	149.67	213.00	184.67	213.0	240.33	248.00	252.00	259.33	262.67	265.67	266.33
Mean no. of emergences on a day	0.67	4.66	15.67	48.67	23.66	117.00	39.34	35.00	28.33	18.00	9.33	7.67	4.0	7.33	3.34	3.00	0.66

On 18 April

Days	8	9	10	11	12	13	14	15	16	17	18	19
Total of mean no. emergences on days	2.67	20.00	31.33	89.33	221.33	278.00	306.67	314.33	319.33	323.33	324.00	324.33
Mean no. of emergences on a day	2.67	17.33	11.33	58.00	132.00	56.67	28.67	7.66	5.00	4.00	0.67	0.33

Table 3. Variable analysis of split-plot design on planting dates and cultivars in soybean.

Source	DF	D1 SS	D1 MS	D1 F	Initial emerging date SS	Initial emerging date MS	Initial emerging date F	Emerging potential SS	Emerging potential MS	Emerging potential F	Final emergence percentage SS	Final emergence percentage MS	Final emergence percentage F
Block	2	0.53	0.27		6.692	3.35		66.47	33.24		1311.51	655.75	
(A)	2	71.29	34.64	2376.0**	4589.81	2294.91	396.36**	19226.77	9613.38	45.10**	4564.25	2282.12	148.35
Planting dates EA	4	0.06	0.02		23.16	5.79		852.64	213.16		61.53	15.38	
(B)	8	27.38	3.42	36.42**	147.66	18.46	11.73	13493.43	1686.68	11.73**	34476.84	4309.61	68.83**
Cultivars A-B	16	4.96	0.31	3.30**	46.20	2.89	1.84	4100.35	256.27	1.78	2657.98	166.12	2.54**
B	48	4.53	0.09		75.48	1.57		6902.22	143.38		3005.63	62.61	
Total	80	108.73			4888.99			44641.88			46077.73		

Table 4. Duncan's SSR test of mean of plots on planting dates of soybean.

Emergence parameters	Planting dates	Mean of plots	Significant test 5%	1%
E1	18 April	3.024	a	A
	29 March	1.657	b	B
	9 March	0.741	c	C
Initial emerging date	9 March	28.11	a	
	19 March	15.78	b	B
	18 April	10.07	c	C
Emerging potential	18 April	49.185	a	A
	9 March	17.630	b	B
	29 March	15.481	b	B
Final emergence percentage	18 April	72.07	a	A
	29 March	66.96	b	B
	9 March	54.22	c	C

Table 5. Duncan's SSR test of mean of plots on cultivars of soybean.

Emergence parameters	Cultivars	Mean of plots	Significance test 5%	1%
E1	B6	2.386	a	A
	B3	2.367	a	A
	B9	2.304	a	AB
	B1	1.971	b	ABC
	B5	1.937	b	BC
	B2	1.934	b	BC
	B8	1.623	c	CD
	B4	1.261	d	D
	B7	0.482	e	E
Initial emerging date	B7	21.22	a	A
	B8	18.89	b	B
	B5	18.0	bc	BC
	B1	17.89	bc	BC
	B2	17.89	bc	BC
	B4	17.78	bc	BC
	B6	17.22	cd	KBC
	B3	16.67	cd	C
	B9	16.33	d	C
Emerging potential	B3	42.67	a	A
	B9	40.67	ab	A
	B6	38.67	ab	A
	B2	35.11	abc	A
	B1	28.67	bcd	AB
	B5	26.22	cde	AB
	B8	17.78	de	B
	B4	15.56	e	BC
	87	1.56	f	C
Final emergence percentage	B3	85.56	a	A
	B9	83.33	a	AB
	B6	80.89	ab	AB
	B1	73.78	bc	BC
	B5	69.78	c	C
	B2	67.78	c	C
	B8	57.33	d	D
	B4	42.89	e	E
	B7	18.44	f	F

Table 6. Comparison of mean of plots on emergence parameters of soybean within a planting date.

Emergence parameters	Culti-vars	Mean of plots	5%	1%	Culti-vars	Mean of plots	5%	1%	Culti-vars	Mean of plots	5%	1%
E1	B3	1.24	a	A	B3	2.29	a	A	B6	4.12	a	A
	B9	1.16	a	AB	B9	2.24	ab	A	B3	3.57	b	AB
	B6	0.96	ab	ABC	B6	2.07	ab	AB	B9	3.51	b	AB
	B1	0.88	abc	ABC	B5	1.94	abc	AB	B1	3.33	b	B
	B5	0.85	abc	ABC	B2	1.82	abc	AB	B2	3.27	b	BC
	B2	0.71	abc	ABCD	B1	1.71	bc	ABC	B8	3.14	b	BC
	B8	0.43	bcd	BCD	B8	1.40	cd	BC	B5	3.02	b	BC
	B4	0.32	cd	CD	B4	1.07	c	C	B4	2.39	c	C
	B7	0.11	d	D	B7	0.37	e	D	B7	0.97	d	D
Initial emerging date	B7	33.00	a	A	B7	19.00	a	A	B7	11.67	a	A
	B8	30.00	b	B	B8	16.33	b	AB	B8	10.33	ab	A
	B5	28.67	bc	BC	B1	16.33	b	AB	B2	10.33	ab	A
	B2	28.00	bc	BCD	B4	16.00	b	B	B5	10.33	ab	A
	B1	27.67	cd	BCD	B6	15.67	b	B	B3	10.00	ab	A
	B6	27.00	cde	BCD	B2	15.33	b	B	B4	9.67	ab	A
	B4	26.67	cdc	CD	B5	15.00	b	B	B9	9.67	ab	A
	B3	25.67	cd	CD	B3	14.33	b	B	B1	9.67	ab	A
	B9	25.33	c	D	B9	14.00	b	B	B6	9.00	b	A
Emerging potential	B3	38.00	a	A	B9	29.33	a	A	B6	73.33	a	A
	B9	35.33	ab	AB	B2	29.33	a	A	B3	61.33	ab	AB
	B1	24.00	abc	ABC	B3	28.67	a	A	B2	60.00	ab	AB
	B6	22.67	abc	ABC	B6	20.00	ab	A	B9	57.33	abc	AB
	B2	16.00	bcd	ABC	B5	20.00	ab	A	B1	56.00	abc	AB
	B5	12.67	cd	ABC	B1	6.00	b	A	B8	48.00	bc	AB
	B4	6.67	cd	BC	B4	3.33	b	A	B5	46.00	bc	AB
	B8	3.33	cd	C	B8	2.00	b	A	B4	36.67	c	B
	B7	0	d	C	B7	0.67	b	A	B7	4.00	d	C
Final emergence percentage	B9	84.67	a	A	B3	92.00	a	A	B6	90.67	a	A
	B3	83.33	a	AB	B9	82.00	ab	AB	B9	83.33	ab	A
	B6	72.67	ab	ABC	B6	79.33	ab	AB	B3	81.33	ab	A
	B1	65.33	b	ABC	B1	74.67	bc	AB	B1	81.33	ab	A
	B5	62.00	bc	CD	B5	73.33	bc	AB	B2	79.33	ab	A
	B2	51.33	c	DE	B2	72.67	bc	AB	B5	74.00	b	AB
	B8	35.33	d	EF	B8	62.67	c	BC	B8	74.00	b	AB
	B4	24.67	d	FG	B4	46.00	d	C	B4	58.00	c	B
	B7	8.67	e	G	B7	20.00	e	D	B7	26.67	d	C

Table 7. Correlation between total of mean number of emergences and meteorological factors on days in soybean.

Meteorological factors	Planting dates		
	March 9	March 29	April 18
Mean of air temperature on a day	0.4410	0.5150*	0.9155**
Highest air temperature on a day	0.1816	0.1020	0.8688**
Lowest air temperature on a day	0.3507	0.6646**	0.6547*
Difference of highest and lowest air temperature on a day	-0.1346	-0.4152	0.4950
Mean of temperature on the surface of soil on a day	0.2196	0.0326	0.9562**
Highest temperature on the surface of soil on a day	-0.1715	-0.3200	-0.7837**
Lowest temperature on the surface of soil on a day	0.3888	0.6534**	0.6711*
Difference of highest and lowest temperature on surface of soil on a day	-0.2712	-0.4797	0.4236
Mean of temperature on 5 cm in soil on a day	0.5532*	0.5136*	0.9510**
Mean of temperature on 10 cm in soil on a day	0.6631**	0.5793*	0.9563**
Mean of temperature on 15 cm in soil on a day	0.7841**	0.6498**	0.9535**
Mean of temperature on 20 cm in soil on a day	0.7790**	0.7023**	0.9535**
Mean of relative air humidity	0.4296	0.2301	0.6651*
Sunshine hours	-0.2505	-0.6500**	0.6395*

Table 8. Comparison and correlation of tolerance to cold beween germination in laboratory and emergence in field of soybean.

Cultivar	Seed coat color	GI	EI	GP	FE
B3	Black	1.899	2.367	90	85.56
B1	Yellow	1.810	1.971	80	73.78
B2	Green	1.610	1.934	75	67.78
B5	Green	1.263	1.937	50	69.78
B6	Black	1.166	2.386	57.5	80.89
B4	Yellow	1.097	1.261	57.5	42.89
B9	Black	0.494	2.304	30	83.33
B8	Green	0.394	1.623	12.5	57.33
B7	Yellow	0.269	0.482	12.5	18.44
	R	0.5370	0.5612		

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1) Study on the uses of aphid-resistant character in wild soybean. I. Aphid-resistance performance of F_2 generation from crosses between cultivated and wild soybeans.

Soybean aphid (Aphis glycine) is the most important soybean pest in the northern part of China, and causes great economic losses every year. It not only directly damages the soybean plants and greatly lowers the seed yield, but also transmits many kinds of viruses to produce mottled seed and reduce the visual quality of soybean. The pest is traditionally controlled by chemical spraying methods. Chemical control has many drawbacks, such as increasing the cost of production, inducing the pest to develop resistance to the chemicals sprayed, killing a large number of natural enemies, and contaminating environment and ecological systems. In the long run, developing and growing aphid-resistant or aphid-tolerant soybean cultivars may be the most efficient way of controlling the pest.

Studies on screening and evaluating aphid-resistant soybean germplasms have been performed since the 1970's in China. More than 2,070 cultivated soybean germplasms were evaluated for resistance to soybean aphid, but none of the materials tested was resistant, except for a few glabrous soybeans. Because the glabrous germplasms are usually accompanied by some morphological, physiological, and metabolic defects, and poor agronomic performances, they are very difficult to use in a breeding program.

Wild soybean (G. soja) is a relative of cultivated soybean and it is also one of the host plants for Aphis glycine, so it is possible to identify usable resistant germplasms in this species. We screened about 1,000 G. soja germplasms and identified three of the materials evaluated with high resistance to the pest. The aphid resistance in wild soybeans may be transferred into cultivated soybean genetic background to develop cultivars with resistance.

The purposes of this study are to investigate the possibilities of and the efficient ways for transfer of aphid-resistant genes from wild to cultivated soybean. The patterns of aphid resistance segregating in the F_2 generation of cultivated x wild soybean crosses are described in this report.

Materials and methods: Three crosses C1 (Jilin No. 20 x Gongye 85-32), C2 (Xuan 85-8 x Gongye 85-39), and C3 (Gong 7514-2 x Gongye 85-32), were made in 1987. Jilin No. 20, Gong 7514-2, and Xuan 85-8 are cultivated soybean that are susceptible to Aphis glycine, and Gongye 85-32 and Gongye 85-39 are resistant wild soybeans (G. soja). In 1988, the F_1 plants were planted in the breeding nursery of the Soybean Institute, Jilin Academy of Agricultural Sciences, Gongzhuling, to obtain seeds for F_2 evaluation. The P_1, P_2, and F_2 populations were planted in a net chamber 20 m long x 5 m wide x 2.1 m high, with rows 0.5 m apart and 2 m long. The plants were thinned to 15 cm apart at about V2 stage. Inoculation was made on June 13 by collecting aphid-bearing

plants of Jilin No. 21, taking a small patch of leaf with about 10 to 15 young aphids, with a scissor, and pinning it to the plant to be inoculated. Aphid susceptibility for each plant was evaluated July 11, using the methods described by Feng and Yue (1986, 1988).

The susceptibility scores were rated with the following scales:

Scores	Severity descriptions
0	Plants with very few aphids per plant.
1	Plants grow normally, with aphid numbers fewer than 100 per plant.
2	Plants grow normally, with aphid number estimated between 101 and 300 per plant, which are largely concentrated on the young shoot and leaves.
3	Plants grow almost normally, with many aphids feeding on the young shoots and leaves. The aphid number per plant estimated between 301 and 800.
4	Plants with stunted development and severely wrinkled leaves and young shoots covered with aphids. The aphid number per plant between 801 and 1200.
5	Stunted plants with "oil"-like substances on leaves and shoots, young shoots and leaves are severely wrinkled, and the aphid number per plant estimated over 1201.

The indices of susceptibility (SI) of P_1, P_2, and F_2 were calculated with the following formula:

$$SI(\%) = \frac{\text{(Score x No. of plants corresponding to the score)}}{\text{5 x total number of plants}} \times 100$$

The ratios of resistant vs. susceptible plants and the number of genes for aphid resistance were estimated.

Results: 1. Average resistance performances of P_1, P_2, and F_2 populations: The resistant parents of the three crosses showed susceptibilty scores of about 1.0, indicating that the resistant parents were highly resistant to soybean aphid. All the cultivated parents were highly susceptible, with susceptibility scores larger than 4.0. The results were in accordance with former experiments (Yue Derong, 1988). The average scores and SIs were intermediate but inclined toward the susceptible parents, indicating that the susceptibility was incompletely dominant to resistance (Table 1).

2. Aphid resistance distributions of parents and F_2 generations: All of the plants of cultivated parents showed susceptibility scores of 4.0 or more, while the wild parents were virtually with scores of 1.0, and no immune plant was identified. The data proved that the resistance performances of both resistant and susceptible parents were stable and seldom influenced by environmental factors.

The susceptibility scores of the F_2 plants distributed continuously with a monopeak model but the situations of the peak varied with crosses. The distribution climax of C1 and C2 were at a score of 4.0, while that of C3 was at a score of 5.0. Because most of the F_2 plants were distributed at scores

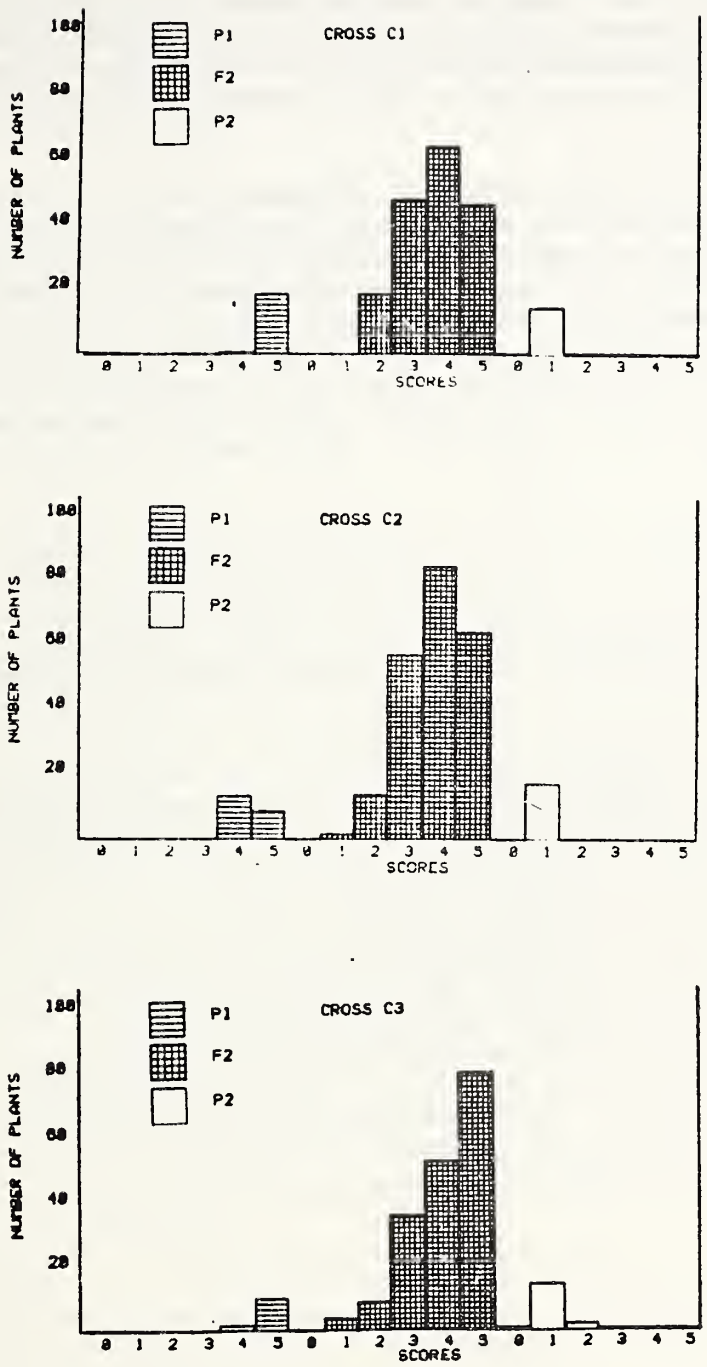

Figure 1. Aphid resistance distributions of F2 plants from three crosses between cultivated and aphid-resistant wild soybeans.

3, 4, and 5, the distributions were all with a non-symmetrical property, suggesting that the character might be controlled by a relatively small number of genes and modified by some other minor genes and environmental factors. If we considered the aphid-resistant character to be a quantitative one, the broad sense heritabilities estimated were 97.0, 85.5, and 85.8% for C1, C2, and C3, respectively.

3. Segregating ratios of resistance to soybean aphid in F_2: According to the performances of resistant and susceptible parents, the F_2 plants were classified into resistant and susceptible categories. Plants with scores 0, 1, and 2 were considered to be resistant, those with scores 3, 4, and 5 to be susceptible. The segregating ratios are shown in Table 2. About 10.2, 7.2, and 7.3% of the F_2 plants were resistant for C1, C2, and C3 crosses, respectively. The actual ratios of resistant vs. susceptible plants were 1 : 8.8, 1 : 12.8, and 1 : 12.8, respectively. The frequencies of resistant plants recovered in the F_2 populations were rather small, and most of the plants were susceptible to the pest.

The resistance segregations of C2 and C3 fit a two-major-gene model, but the fitness of C1 was poor. Considering that the resistant parents of C1 and C3 are the same (Gongye 85-32), the F_2 data of the two crosses were pooled, and they also fit the two-gene model ($X^2 = 3.32$, $P > 0.05$). Therefore, the aphid resistance of Gongye 85-32 and 85-39 might be controlled by two independent recessive genes.

Discussion: 1. Suitable crossing patterns for transfer of aphid resistance genes: Wild soybean usually has many undesirable characteristics, such as prostrate habit, shattering, small and hard seed, poor visual quality, susceptibility to diseases, etc. In the aphid resistance breeding program we only want to introduce the resistance character into cultivated genetic background. Selections after a single cross favor the resistance character, but the recovery of agronomic characters in each cycle of selection will surely be small. To improve recovery of agronomic performance, backcrossing is often used with the cultivated soybean as recurrent parent. The possibility of losing resistance genes increases with the number of backcrosses made in each cycle of selection, especially when the resistance is controlled by recessive genes. It is recommended that one cross and one backcross be made in each cycle of selection to guarantee that the resistance character be maintained and moderate agronomic progress be made.

2. Optimum selection generations for aphid resistance: The aphid resistance character may be controlled by recessive genes, on the basis of this study. So the frequencies of resistant plants in the F_2 generation are very small. Theoretically, the frequency of resistant plants increases with the advance of generations. When the resistance is controlled by two recessive genes, the frequencies of resistant plants in the F_2, F_3, and F_4 populations will be 6.25, 14.06, and 19.14%, respectively. Therefore, selections on resistance and agronomic characters can be made simultaneously in later generations, such as F_3 or F_4, among relatively large numbers of resistant plants, to improve breeding efficiencies.

3. Mechanisms of aphid resistance: Wild soybean differed greatly in many morphological, physiological, and ecological characteristics from those of cultivated soybeans. If aphid resistance is related to these wild soybean

characteristics, it will be difficult to use. Although most of the F_2 plants from crosses between cultivated and wild soybeans were similar to the wild parents morphologically, most F_2 plants tended to be susceptible. It can be concluded that the resistance in wild soybean is not necessarily related to the wild soybean characteristics, such a prostrate habit, branching, small and hard seed, shattering, etc.

4. Possible ways for transferring aphid resistance genes from wild soybeans: Transfer of aphid resistance genes from wild soybeans with the traditional crossing and backcrossing methods is a time-consuming process. Using molecular genetic management techniques, taking the resistance genes carrying DNA segments from the wild soybean donor and introducing them into cultivated soybean genetic background may be an efficient way to develop aphid-resistant cultivars. We are trying to investigate the possibilities of genetic transformation.

Table 1. Average resistance of parents and F_2 generations.

Crosses	Generations	Susceptibility scores ($X^2 \pm S$)	SI # (%)
C1	P_1 Jilin 20	4.94 ± 0.23	98.9
	P_2 Gongye 85-32	1.00 ± 0.00	20.0
	F_2	3.76 ± 0.94	75.1
C2	P_1 Xuan 85-8	4.41 ± 0.50	88.2
	P_2 Gongye 85-39	1.00 ± 0.00	20.0
	F_2	3.88 ± 0.93	77.6
C3	P_1 Gong 7514-2	4.83 ± 0.39	96.6
	P_2 Gongye 85-32	1.10 ± 0.38	22.0
	F_2	4.08 ± 1.02	81.6

Indices of susceptibility

Table 2. Aphid resistance segregating ratios of crosses between cultivated and wild soybeans in F2 generations.

Crosses	Generations	Total plants	Resistant* No. of plants	Resistant* Frequencies	Susceptible No. of plants	Susceptible Frequencies	Fitness^ X^2	Fitness^ P
	P1	19	0	0.000	19	1.000	-	-
C1	P2	14	14	1.000	0	0.000	-	-
	F2	176	18	0.102	158	0.898	4.10	>0.025
	P1	22	0	0.000	22	1.000	-	-
C2	P2	17	17	1.000	0	0.000	-	-
	F2	221	16	0.072	205	0.928	0.22	>0.50
	P1	12	0	0.000	12	1.000	-	-
C3	P2	22	22	1.000	0	0.000	-	-
	F2	179	13	0.073	166	0.927	0.16	>0.50
C1 + C3	F2	355	31	0.087	324	0.912	3.32	>0.05

* Plants with susceptibility scores 0, 1, and 2 are classified as resistant.

^ Theoretical ratio tested is 1 resistant vs. 15 susceptible.

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1) The effect of blend to increase soybean yield.

In 1989, 38 blends with different combinations and ratios of four soybean varieties were planted in Harbin, to study the effect for increasing soybean yield.

The varieties used as blends, and check, were 'Heinong 29', 'Heinong 33', 'Heinong 34', and 'Hefeng 29'. The ratios of combined blends were 1:1, 1:3, and 3:1 for two-variety combinations; 1:1:1, 1:2:3, 3:2:1, and 2:3:1 for three-variety combinations; 1:1:1:1, 1:2:1:2, 2:1:2:1, and 1:2:2:1 for four-variety combinations.

(1) How many varieties does it take to make a blend with high yield?

To get good yield, the number of varieties combined to blend is not too much. In general, two varieties are best (Table 1). There were 18 blends combined with two varieties in our experiment; their average yield was higher than check yield by 6.4%, and six of them were significantly higher than the check.

The average yield of blends combined with three or four varieties was somewhat lower than the check, even though there were few blends that had higher yield than the check.

Table 1. Yield of blends combined with different variety numbers.

No. varieties to make blend	Av. blend	Yield check (g)	No. blends	No. blends with high yield	Compare blend yield with check (%)
2	584.3	549.4	18	10	106.4
3	529.5	563.4	16	7	94.0
4	533.2	549.3	4	2	97.1

Although the average yields of blends combined with three or four varieties were similar, the average yield of blends combined with two varieties was significantly higher.

2. Is there some difference among blends combined with different varieties?

In the case of two-variety combinations, the yield of blends from Heinong 29 and Hefeng 29 with any combination ratio was the lowest. But the yield of

blends from Heinong 33 and Hefeng 29 was highest. It was significantly higher than the average yield of check by 22.1-47.7% (Table 2).

Table 2. Yield of two-variety blend from different soybean varieties.

Blend combination*	Average blend	Yield check (g)	Compare blend with check Range	Average
N29 + N33	531.8	607.4	69.1-109.7	87.6
N29 + N34	591.5	603.7	95.2-101.0	98.0
N29 + F29	509.2	549.7	76.8-104.5	92.6
N33 + N34	578.0	549.0	91.7-114.7	105.3
N33 + F29	734.3	495.2	133.9-164.8	148.3
N34 + F29	560.0	491.1	107.7-120.6	114.0

* N29 = Heinong 29; N33 = Heinong 33; N34 = Heinong 34; F29 = Hefeng 29.

These results show that there is some positive or negative relationship between the two varieties combined for blend during the growing period. In the case of positive relationship, two varieties complement each other. In the case of negative relationship, two varieties restrict or repress each other. Therefore, the best suitable combination is needed to get high yields by using blends.

3. What is the best ratio for a two-variety blend?

In the case of blends combining two varieties, if the ratio of the same two original varieties is different, there will be a difference in the effect on yield increase. Generally, 1:1 is the best ratio to get the best yield. There were six blends combined with two varieties in a 1:1 ratio in this experiment. Their average yield was higher than the check by 6.6%. Four of them were higher than the check by more than 10%. Twelve blends were combined with two varieties by a 1:3 ratio. Their average yield was almost the same as the check. Less than half of them showed yield that was higher than the check. But there were two blends of this kind of combination ratio that had the highest yield in the whole experiment.

We can say that, in general, blends combining two varieties by a 1:1 ratio are usually better, although they may not be the best. To get a blend with the highest yield, it is not only necessary to select the best combination of varieties, but also the best combination ratio.

Table 3. Difference in yield among two-variety blends with different ratios.

Ratio to make blend	Blend yield (g)	Check yield (%)	Compare blend with check (%)
1 : 1	607.6	549.3	110.6
1 : 3	570.2	520.8	109.5
3 : 1	575.0	578.0	99.5

4. Our results were obtained in Harbin in 1989, a dry weather climate, in black soil with a high level of fertilizer. This will affect the results of research on use of blends. But how will different environments and different years effect results? What will be the potential to get high yield and possibilities for stability of yield? All of these questions must be studied continuously and in depth.

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2) Brief on soybean mutation breeding.

The United States, East Germany, and Japan have carried on research in soybean mutation breeding in the past, with great progress. Our academy began to study soybean mutation breeding in 1958, and we have selected 10 soybean varieties, which are planted on more than 3 million hectares. This paper introduces the technique of soybean mutation breeding that we have studied, and the effect of selection.

I. Study of technique of soybean mutation breeding.

Methods of induced mutation: The main problem of induced mutation is the suitable dose of mutation treatment. In the past, it was thought that the LD50 was the suitable dose of induced mutation, but we have noticed that the suitable dose is determined by biological effects on the M1 generation and the genetic effect on the progeny. On the basis of two to six years experiment, with 4 to 30 treatments, we suggest that available treatments of inducing mutation are shown in Table 1.

Radiosensitivity: Radiosensitivity determines the M1 effect, progeny mutation frequency, and variance range. Biological and environmental factors affect the radiosensitivity to physical and chemical mutagens in soybean. The survival rate ranged from 22 to 73% in the same treatment, due to different genotypes. Differences of radiosensitivity of F1 generations depended upon the parents' genotypes. In environment, for example, dry seeds treated by r-ray with 90 Gy were stored in oxygen for 18 hours at room temperature. Survival rate of the M1 generation decreased from 69 to 22%, normal plant rate and semi-sterile plant rate increased 10.77% and 6.00%, respectively, and the sterile plant rate increased 17.12%.

Relationship between plant type of M1 generation and valuable mutations of progeny: Of valuable mutations such as early-maturation, strong stem, high yield, large seed size, 60% were derived from normal plants of the M1 generation, 30% were derived from semisterile plants, and less than 10% were derived from other types of plants. Experiments showed that the effect of selecting from progeny of M1 normal plants was better than those from offspring of other plant types, for selecting high yield mutants, particularly.

Effects of storage on soybean seeds treated with r-rays: Our study indicated that there were differences in 1) survival rate ($p<0.01$), 2) number of sterile plants ($p<0.01$), 3) M1 seedling height, 4) chlorophyll aberrations, 5) plant height and seed size in the M2-M3 after soybean seeds were irradiated with Co^{60}r-ray and stored for a different number of days. The results showed that effects of storage appeared in the M1 generations as well as in the M2 and M3. We found that 16 days after radiation there was the changing point of some mutations in the M2. For example, this treatment group had the highest chlorophyll aberration frequency, the highest plant height, the smallest seed size in the M3, and early-maturing frequency decreased as the days increased. In order to get better effect of induced mutation, a breeder should use available treatment time.

II. Application of the technique to soybean induced mutation breeding:

We used stable varieties or lines in the experiment and found that physical and chemical mutagens can broaden mutations in maturity, plant height, branching, seed size, single-plant yield, protein and oil content to increase the chance of selecting excellent mutations. By combining methods of mutation breeding with cross breeding, we also can get abundant gene recombination, increased mutation frequency, and variation; we can also obtain some mutants that we couldn't get with only one of these methods. The method of combining mutation breeding and cross breeding included crossing with mutants as one or two parents, and treating seeds of the F_1 and F_2 generations with mutagens.

Development of 11 varieties and several lines by induced mutation breeding.

High oil-content varieties such as Heinong 6, Heinong 8, Heinong 16, Heinong 31 which average 22-23% oil content increased 1.00 to 2.71% over untreated control. They also have stable yields, wide adaptability, as the cultivated area was more than 1.05 million hectares. There are a few varieties, for instance Heinong 5, Heinong 26, Heinong 28, et al., that have tall plants, strong stems, high yield, and have been planted on 2.02 million hectares. Now we have obtained many mutants with disease resistance that are high yielding in uniform test, and are to be used in production.

We can create mutants with special value by inducing mutation; for example, tolerance to alkaline or saline soil. Super early mutant LF 464 has a period from germination to maturity of 100 to 103 days, 25 to 30 days shorter than the untreated check. Its chlorophyll content is reduced 30% from the primary variety, with photosynthetic efficiency increased by 16%, and it had a yield of 2205 kg/ha. These two mutants did not occur in soybean genetics in the past.

Creating high protein content mutants and high total content of protein and oil.

In traditional breeding programs there is a negative correlation between protein and oil content; protein content cannot be increased if oil content does not change. We obtained higher protein content mutant LF 837 by radiation. The average protein content is 46.66% in three years; its maternal parent FengShou No. 11 (derived from progeny of radiation) has 39.1% and paternal parent Fiskeby has 39.55%. We also obtained a mutant with protein content of 47 to 48% and oil content of 18 to 19% from the M3 generation of LF 837 treated with EMS.

LF 837 that came from the F_2 of Harosoy 63 x QunXuan No. 1, treated with thermal neutrons 8x10"^{N}/cm2 has an average protein content of 43.5% for three years, 2.04% and 3.14% higher than its parents, respectively. Oil content of 20.79% was 0.29% and 1.29% higher than its parents. The negative correlation between protein and oil content seems to have been changed.

Induced mutation breeding has been an effective method of soybean improvement. Our further studies include how to increase mutation frequency and selection effect.

Table 1. Suitable method for induced soybean mutation.

Treatment	Dosage and concentration	Condition and Time
r-rays	80 to 120 Gy	dry seed dosage rate 60-80 r/min
Thermal neutron	3x10"-8x10"^{N}/cm2	1.6x10^{10} ^{N}/cm^2 second
^{32}PB-rays	40-50uc/seed 40-50uc/ce	germinated 16°C 24 h bud 24 h, buried under land
EMS	0.2-0.4%	direct soaking 24 h, pH 7
NaN_3	1-5 mM	direct soaking 24 h, pH 3

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1) A media system to enhance efficiency of plant regeneration in Glycine max

Introduction: Soybean is an important commercial crop for oil and protein production in the world. In recent years many non-traditional techniques have been developed for soybean improvement. To effectively utilize new techniques for high frequency of regeneration systems, in which a large share of calli population is competent to regenerate, it is especially necessary for *in vitro* transformation or selection.

It has been proved that large-seeded grain legumes possess an unusual hindrance to plant regeneration (Hammatt et al., 1986). Since the 1970's much attention has been paid to somatic embryogenesis and plant regeneration in soybeans, a legume. Until the past five years, soybean regeneration via embryogenesis was successful in a few laboratories (Lazzeri et al., 1985; Ranch et al., 1985; Barwale et al., 1986; Hammatt et al., 1987, and Komatsuda, 1988), but the efficiency of regeneration still remained low. Although sugar had been emphasized to take a main role for somatic embryogenesis in soybeans (Lippman and Lippman, 1984; Lazzeri, 1986), much less attention was focused on the influence of sugar content to maturation and germination of somatic embryos (Lazzeri, 1986). An attempt of our present work, therefore, was to determine the most optimal concentration of sucrose in maturity medium to increase the efficiency of regeneration.

Materials and methods: Seeds of the cultivar 'Viking' were obtained from the National Agrobiological Resources Institute in Japan. Immature pods containing seeds 2.5-3.5 mm long were harvested and stored at 4 C for 48 h. Embryos then were excised from seeds, asceptically, divided into two cotyledonary explants and incubated in tubes with 10 ml of gelrite-solidified medium (MSB). The embryogenesis medium contained MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968), 0.5% sucrose, 10 mg/l NAA, 0.22% gelrite, and was adjusted to pH7 before autoclaving 15 min at 120 C, 120 kpa).

Secondary culture was conducted after 4 weeks. The formed somatic embryos with calli were transferred into media A and B, respectively. Medium A consisted of MSB plus 1 mg/l NAA and 0.5% sucrose and B consisted of MSB plus 1 mg/l NAA and 3% sucrose. In this section, embryos were cultured for 2 weeks. Further, tissues were transferred into six kinds of medium that belonged to two media systems, as shown in Figure 1, and subcultured each 2 weeks, with examination of fresh weight and color of somatic embryos.

Five weeks later, when somatic embryos matured, they were transferred into 1/2 MSB medium with 0.001 mg/1 GA3 plus 1% sucrose (according to Takao Komatsuda, 1988, unpublished) and cultured for 4 weeks. Every 2 weeks, frequency of germination was recorded.

In this experiment, completely randomized design was adopted with two replications and six treatments.

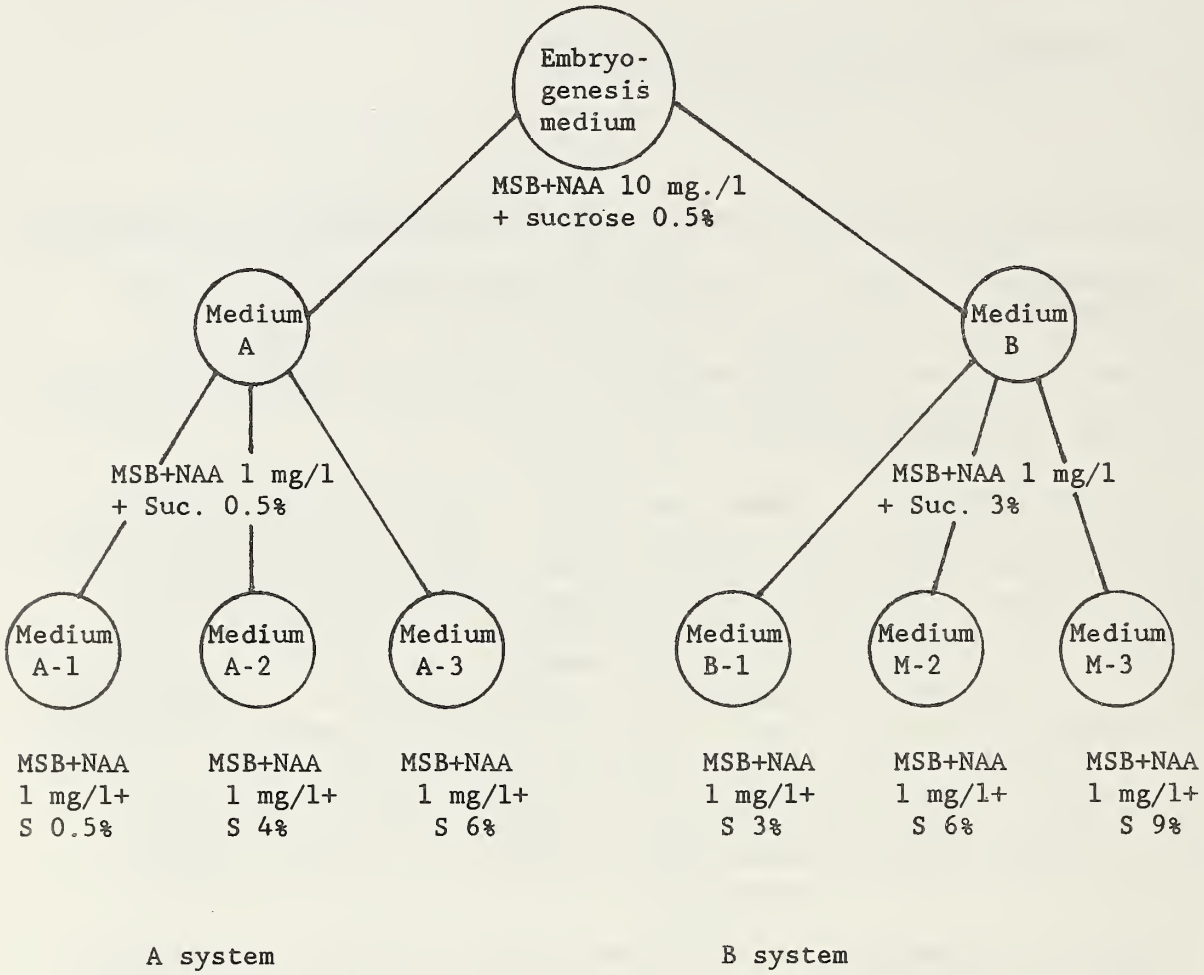

Figure 1. Two Media Systems

Preliminary results and discussion

Maturity Culture: Somatic embryos cultured in the medium B system grew more quickly and their color gradually changed from green to yellow, while in the medium A system, the somatic embryos grew much more slowly and color varied from water-green to pale-green. Of the traits counted, fresh weight of somatic embryos gave the highest increase, and length of hypocotyl elongation, very gently (Table 1).

Time(wks)	4	6		8		10		11		13+	
Media / Traits	MSB	A	B	Sys A	Sys B	Sys A	Sys B	Sys A	Sys B	Sys A	Sys B
Length of hypocotyl (mm)	0.4	0.5	1.3	1.5	1.7	1.9	2.2	2.5	2.7	4.1	5.5
Fresh wt. of embryos (mg)	0.6	0.7	1.0	2.9	4.1	4.3	6.0	4.7	6.0		

^ - Media system.
+ - Transferred into 1/2 MSB for 2 weeks.

as sucrose concentration in a medium was increased, the average fresh weight of somatic embryos was promoted simultaneously, but 9% concentration of sucrose was the upper limit. A different effect was observed between media A and media B systems. In the B-2 medium, containing 6% sucrose, somatic embryos gave the highest fresh weight.

Germination culture: After culture in germination medium for 2 weeks, the length of hypocotyl, diameter of hypocotyl, frequency of germination and ratio of rooting were counted. The highest frequency of germination occurred in somatic embryos cultured in B-2 medium (44.14%) (Table 2) and the frequency increased

Table 2. Performance of traits on embryos derived from two media systems after tranfer to 1/2 MSB for 2 weeks.

Media / Traits	A-1	A-2	A-3	X	B-1	B-2	B-3	$\bar{X}$
Germination (%)	11.75	16.50	22.19	16.8	19.23	44.14	32.33	31.9
Ratio of rooting	2.50	14.40	45.27	20.7	34.50	69.38	49.77	51.2
Diam. of hypocotyl (mm)	0.83	0.96	0.98	0.92	1.00	1.15	1.17	1.11

continually, reaching 60.5% at 105 days from incubation in embryogenesis medium. It was proved by variance analysis that a significant difference in frequency of germination existed between two media systems including four sucrose concentrations (F value = 41.24, $P<0.01$).

In our experiment, an obvious morphological feature of germination seemed to be a rapid elongation of the hypocotyl. From the data in Table 2, it could be inferred that after transfer into germination medium (1/2 MSB) the length of the hypocotyl on somatic embryos had outstanding increase. Evidence obtained from correlation analysis also showed that frequency of germination and length of hypocotyl gave the biggest positive correlation coefficient, $r=0.92$ ($P<0.01$).

According to the above results, a media system suitable for high frequency of plant regeneration was:

```
                          4 w            2 w.            4.5 w.
Embryogenesis medium ----->Medium B ----->Medium B-2------>
      (MSB)

                          2-4 w.
                 1/2 MSB-------->Germination
```

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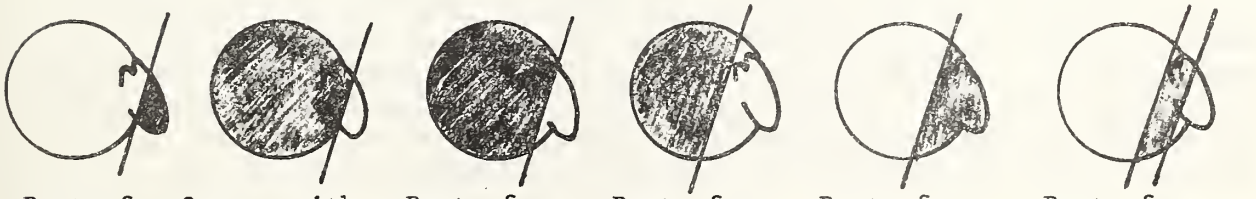
2) Comparison of embryogenesis efficiency on eight portions of immature embryos in Glycine gracilis

Introduction: In recent years a great advance has been achieved in soybean in vitro culture. Especially, somatic embryogenesis and plant regeneration from immature embryos have succeeded in a few laboratories (Lazzeri, 1985; Ranch, 1985; Barwale, 1986; Hammat, 1987; Komatsuda, 1988). As Agrobacteria vectors were used to foreign gene transformation in soybeans, searching a convenient path for absorbing genes into isolated cotyledonary tissue appeared to be necessary. In this case an important work was to evaluate embryogenesis efficiency from different portions of immature embryos. In melon, different response to embryogenesis had been observed among six kinds of isolated positions on immature embryos (Homma et al., 1989). However, less information is available in soybeans. The present study was undertaken to analyse the competence of somatic embryogenesis induced from eight kinds of immature embryo explants in soybeans.

Materials and methods: Seeds of Kou 502 (G. gracilis) supplied by National Agrobiological Resources Institute in Japan were planted in the greenhouse. Immature pods containing seeds 2.5-3.5 m long were stored at 4 C for 48 h, and then surface-sterilized pods by immersing for 30 sec in 70% ethanol followed by 10 min immersion in 2.5% commercial sodium hypochlorite solution with one drop of Triton X-100. Further embryos were extracted from seeds and cut to eight shapes of explants as shown in Figure 1. Explants were placed on 10 ml of embryogenesis medium containing MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968) 0.5% sucrose, 10ml/l NAA, 0.22% gelrite, at pH 7.0 before autoclaving, and cultured under dim light (ca. 50 Lux) at 25 ± 2 C. The experiment was designed by using completely randomized method with two replications. Characters on calli and embryogenesis were examined at 10 days and 30 days after being incubated, respectively.

one cotyledon (co)
with radicle (ra) and shoot apex (sh)

whole embryo
with pair of co



Part of ra (A)

One co with part of ra & whole sh (B)

Part of co with sh (C)

Part of co with no ra & sh (D)

Part of co with whole ra & sh (E)

Part of co with part ra & whole sh (F)

Results and discussion: Ten days after culturing, distinct calli were formed and could be distinguished as various types by shape and color. Numbers of cotyledons inducing no callus varied to a great extent in eight kinds of explants. That was D>A>F>C>B>E>G and H. On the other hand, brown and pale-brown calli were expressed as H>G>B>E>A>F>C>D. In this period no somatic embryo was generated on callus.

Table 1 showed the embryogenesis result counted at one month after incubation. A high embryogenesis efficiency was achieved from B, G, and H explants, a middle value occurred at C explant, and low values took place on D, E, and F explants, while A explant gave no embryogenesis efficiency. Depending on variance analysis, a significant difference was obtained among eight explants in embryogenesis efficiency (F value=9.15; P<0.01).

Table 1. Embryogenesis efficiency in eight kinds of explants. (1990, Japan).

Traits / Explants	Embryogenesis# efficiency	L.S.D. value P<0.05	L.S.D. value P<0.01
A	0.00		
B	3.53		
C	2.08		
D	0.38	0.72	1.43
E	1.07		
F	0.36		
G	4.11		
H	3.08		

Cited from Lazzeri, 1988.

High negative correlation was observed between ratio of no callus plus white callus and embryogenesis efficiency (r=-0.74; P<0.01); in contrast, similar positive correlation was possessed by ratio of brown callus and embryogenesis efficiency (r=0.72; P<0.01). This seemingly indicates that formation of brown callus was a major factor to induce somatic embryos; however, the position where brown callus was generated was also important to embryogenesis. As explant A without cotyledon and shoot apex, 40% of the explants could produce brown callus, but no embryogenesis was acquired from them.

Based on the above results, it suggested moderate section of radicle and whole shoot apex were imperative to obtain a large number of somatic embryos. However, usually somatic embryos emerged from cotyledon place, moderate part of cotyledon was also necessary to embryogenesis. To aim at DNA transformation, B and C explants possibly provided a beneficial effect, since they possessed higher embryogenesis efficiency as well as an easier thoroughfare for transferring foreign genes into embryo tissue.

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1) Evaluation of soybean varieties using nearest neighbor analysis and the estimation of genetic improvement as a result of registration of new varieties.

The present analysis was carried out with the data of the Institute of Agricultural Certification. The experiments took place at 11 locations in Hungary, using the same methods and were evaluated with ANOVA.

For further investigation we tried to make use of a method capable of evaluating data of incomplete blocks and tends. The method of Nearest Neighbor Analysis (NNA), elaborated by Wilkinson (1983) and developed further by Schwarzbach (1984, 1985), offers a good opportunity to find the most probable order of varieties even in such cases.

The method is based on the analysis of neighboring plots and makes it possible to reduce heterogeniety within a given variety. In the first step the NND_a/Nearest Neighbor Difference/value, i.e., the difference from the average of the neighboring plots is calculated. In the second step the NND_e value, the difference, expected on the basis of the average values of varieties is received. The average of varieties is corrected with the difference of the actual and the expected values, and this calculation is repeated several times to decrease the effect of errors. For the calculation of the components of error, Wilkinson et al.,(1983) and Schwarzbach (1984) can be referred to. Genetic improvement was calculated on the basis of linear trends, using a modified method of Szabo et al. (1987).

Out of the varieties, 'Evans', ISZ-15, and ISZ-16 were involved in the experiments of all of the 11 years under study. For the rest of the varieties we have data from 3 to 9 years, depending upon their time of registration.

As it can be seen in Table 1, the same varieties received the last five places, according to all three methods. However, the first five differed significantly according to the order of productivity.

On the basis of the average A-1, the variety 'Crusader' is placed first, but according to the other two methods it is only calculated fourth, because in evaluating the order of varieties we did not take into consideration that the given variety was only used in the experiment of four good years.

If, following the principle of comparing equal to equal, we compare the yield of the last four years, 'Eszter' is placed first, BS-31 second, and S-1346 third. This evaluation corresponds to the present evaluation method of

the Institute of Agricultural Certification. Its disadvantage is, however, that it can only consider the last three to four years of a longer period.

According to the order of varieties determined by the NNA method, the most productive variety is S-1346, to be followed by BS-31 and Eszter. According to the two other methods, BS-31 is equally ranked second; thus, further studies are needed to decide the question of priority. In order to do so, we determined the average for eight years of S-1346 that was 2800 ± 106.4 kg/ha and compared it to the average for eight years of the Eszter soybeans variety (2771 ± 174.7 kg/ha). Following the averages for eight years of the two varieties it can be found that in the given period S-1346 was the most productive variety and this fact seems to support the order of varieties determined by the NNA method.

Using the NNA method the registered soybean varieties had their protein content between 38.4 and 40.4 % (expressed in per cent of dry material). BS-38 had the highest protein content (40.4%) followed by CM-048 (40.1%) and 'Imola' (39.8%). The lowest protein content belonged to S-1346 (38.4%) and Evans (38.6%). The average of three varieties (Evans, ISZ-15, and ISZ-16) was 34.3% of protein in 1981 and 42.9% in 1986. This significant difference in the effect of years makes it difficult and unreliable to evaluate the obtained difference of varieties.

We did not find a reliable difference with respect to the oil content of the soybean varieties.

Following the NNA method the highest oil content was found in Eszter (22.0%) and BS-31 (21.8%). The least oil was found in CM-137 (20.5%) and ISZ-16 (21.0%) In this case, too, the effect of the year was also very significant. The average of the above three varieties with respect to their oil content was 19.1% in 1979, and 23.2% in 1981. The protein content (y) and the oil content (x) of the varieties had the relation of $y = 52.81 - 0.64x$. The correlation coefficient between the variables was $r = 0.511$.

As an effect of the NNA method, the average yield of varieties cultivated earlier was corrected upwards, whereas that of varieties cultivated under more favorable weather of the last years and using improved agrotechniques was corrected downwards. In spite of this, the orders of varieties obtained on the basis of averages as well as the NNA method do not differ significantly, thus showing that the improvement in yield could be understood as the consequence of the change of varieties.

Table 2 shows that genetic improvement as a result of the change of varieties was 16.1 kg/ha/year in terms of seed yield, that makes 50.8% of the average realized annual growth in yields (31.6 kg/ha/year).

As for protein content, a genetic improvement of the newly registered varieties could not be attested. In the end of the examination the protein content of new varieties were less than that of the standard varieties. In the given period (1977-87) the raw protein content of the standard varieties rose by 3.33%, the average protein content of the others varieties under study rose by 2.63%.

In the given period the oil content of the newly qualified varieties

Table 1. Yield (kg/ha) of standard and newly registered soybean varieties and the comparison of the orders of varieties obtained in different ways.

Variety	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	A-1	R-1	A-2	R-2	A-3	R-3
Evans	2540	2340	2476	2548	2509	2853	2200	3352	2080	2464	2869	2566	7	2691	7	2592	7
ISZ-15	2446	2455	2537	2284	2264	2678	1713	3140	1863	2315	2705	2400	8	2506	8	2425	8
ISZ-16	1809	2177	2182	2140	2043	2421	1650	3030	1816	2052	2452	2161	10	2338	10	2186	10
S-1346			3005	2830	2738	2990	2513	3400	2465	2610	2851	2822	2	2832	3	2822	1
Eszter				2387	2774	3233	2270	3682	2280	2649	2892	2771	5	2876	1	2785	3
BS-31						3085	2468	3360	2392	2628	2993	2821	3	2843	2	2790	2
McCall						2588	1645	3040	1857	2365	2635	2355	9	2474	9	2320	9
Imola							2027	3456	2174	2695	2700	2610	6	2756	6	2655	2
CM 048								3488	2123	2582	2874	2767	4	2767	5	2689	5
Crusader								3429	2377	2549	2944	2825	1	2825	4	2764	4
Average	2265	2324	2250	2438	2466	2835	2061	3338	2143	2491	2792						

Remarks: A-1: Average obtained from all data of varieties. R-1: Order of varieties belonging to it.
A-2: Average obtained from all data of last 4 years. R-2: Order of varieties belonging to it.
A-3: Average obtained using NNA method. R-3: Order of varieties belonging to it.

Table 2. Improvement in yield resulted from the registration of new varieties in 1977-1987 and the change in oil and protein content (in % of dry matter).

Entries	Average yield corrected on basis of the trend			Change in 1 year	Correlation coefficient
	1977	1987	Difference		
Seed Yields					
Registered varieties	2360.41	2676.50	316.09	31.60	0.2897
Standard varieties	2298.10	2453.50	155.40	15.40	0.1418
Genetic improvement			160.69	16.07	
Protein content (% of dry matter)					
Registered varieties	37.13	38.76	2.63	0.26	0.3258
Standard varieties	37.6	40.39	3.33	0.33	0.3760
Genetic improvement			-0.70	-0.07	
Oil content (% of dry matter)					
Registered varieties	21.28	21.46	0.18	0.018	0.0406
Standard varieties	21.32	21.42	0.10	0.010	0.0538
Genetic improvement			0.08	0.008	

rose by 0.18%, whereas those of the standard varieties rose by 0.10%. The difference between the two values can be understood as a result of genetic improvement, but its low value and the significant effect of years do not make it statistically relevant.

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1) Somatic hybridization of Glycine species: The use of flow cytometry for isolation of heterokaryons.

Introduction: Wild perennial Glycine species have characteristics of potential agronomic importance. These include daylength neutrality, tolerance to heat, drought, cold (Marshall and Broué, 1981) and salinity (Newell and Hymowitz, 1982), and resistance to soybean cyst nematode (Riggs and Hamblen, 1962, 1966), yellow mosaic virus (Singh et al., 1974), powdery mildew (Mignucci and Chamberlain, 1978) and rust (Burdon and Marshall, 1981). Somatic hybridization, involving protoplast fusion, could facilitate gene flow between the cultivated soybean (G. max) and its wild relatives by circumventing inter-specific sexual incompatibility (Davey and Hammatt, 1987).

The production of somatic hybrids involves the exploitation of protoplast-to-plant technology. Although plant regeneration from seedling protoplasts has been reported for Chinese cultivars of Glycine max (Wei and Xu, 1988), a reproducible method of plant regeneration from isolated protoplasts of Western varieties has not been described, despite extensive research (Davey et al., 1988). Attempts to produce somatic hybrid plants, whether in Glycine or in other genera and species, is dependent on plant regeneration by at least one of the fusion partners. Previous research at Nottingham has involved the development of procedures for reproducible regeneration of plants from protoplast-derived tissues of wild Glycine species, including G. argyrea (Hammatt et al., 1989a), G. canescens and G. clandestina (Davey and Hammatt, 1987).

A problem fundamental to somatic hybridization is the selection of heterokaryons from the unfused protoplasts and homokaryons. As summarized by Hammatt et al. (1990), there are many advantages to be gained by isolating heterokaryons immediately post-fusion, ready for culture into somatic hybrids. This procedure has been assisted by the development of dual fluorescence labelling of protoplasts to allow identification of the heterokaryons (e.g., Patnaik et al., 1982). Protoplasts from light-grown Glycine seedlings contain chlorophyll which emits red fluorescence upon absorption of blue light. In contrast, protoplasts from dark-grown Glycine seedlings are colorless, but can be labelled green by including fluorescein diacetate (FDA) in the enzyme mixture during protoplast release. Heterokaryons formed between these protoplast populations fluoresce simultaneously red and green.

Although putative somatic hybrid plantlets have been produced from bifluorescent heterokaryons of G. argyrea G1626 x G. canescens G1171 using micro-manipulation (B. Jones, N. Hammatt and M.R. Davey, unpublished data), this procedure is time consuming, requires considerable manual dexterity, and usually results in the recovery of

relatively small numbers of heterokaryons. Flow cytometry is an automated technique which can be employed for the recovery of large numbers of heterokaryons, based on bifluorescent emissions from these protoplasts. This technique is applicable not only to Glycine, but to an increasing range of plant species.

Heterokaryon sorting: Flow cytometric techniques were originally developed for use with human cells which have diameters of 5-15 um. In contrast, plant protoplasts can be as large as 95 um and require special handling because of their fragility. Extensive modifications to the EPICS V flow cytometer (Coulter Electronics Ltd., Luton, England) located in the Plant Genetic Manipulation Group at the University of Nottingham, have facilitated the reliable sorting of plant protoplasts.

Following protoplast fusion, the mixture of heterokaryons, homokaryons and unfused parental protoplasts is passed through a fine needle (100 um internal diameter) into a fast flowing stream of liquid. By adjusting the pressure differences, the protoplasts are focussed into a narrow core, the path of which is intersected by light from an argon-ion laser. This arrangement is adjusted so that only one protoplast is in the path of the laser beam at any point in time, and results in the production of scattered laser light (blue) and fluorescent emissions (green and red). A series of glass filters separates the light into its different colors; the intensity of each color is then quantitated by a separate photomultiplier tube.

Information for each protoplast is collected by the computer of the flow cytometer, and assimilated into a form suitable for display. In this case, a graph of green fluorescence is plotted against red fluorescence (Fig. 1). Thus, in Figure 1A, the FDA-labelled G. argyrea dark-grown seedling hypocotyl protoplasts fluoresce green, while in Figure 1B, G. canescens light-grown seedling cotyledon protoplasts exhibit red autofluorescence. Figure 1C represents a physical mixture of the two protoplast populations before fusion. In Figure 1D, three distinct populations can be distinguished after fusion treatment. Specifically, unfused G. argyrea hypocotyl protoplasts (box 1), unfused G. canescens cotyledon protoplasts (box 2) and heterokaryons (box 3). A population of yellow/orange fluorescent polystyrene spheres was used to calibrate the instrument. These can be seen in the centres of graphs A and B.

The stream is vertically oscillated by a piezoelectric (quartz) crystal to form small uniform droplets. Normally, the droplets are given a small electric charge, and are deflected to one side by two charged plates. When a heterokaryon is identified, the droplet containing the fusion product is left uncharged. Consequently, it is not deflected, and descends into the well of a microtitre plate containing culture medium.

Characteristics of tissues derived from flow-sorted Glycine heterokaryons: The results, to date, of the flow cytometry of Glycine protoplasts are summarized in Table 1. Previously, the induction of shoot-like structures on somatic hybrid callus derived from flow sorted heterokaryons of G. max cv. HP-20-20 x G. canescens G1171 has been reported (Hammatt et al., 1988, 1989b). However, these shoot-like structures have, to date, failed to develop into plants. Although

protoplasts of the wild Glycine partner undergo plant regeneration, failure of heterokaryon-derived material to produce plants may relate to the dwarf nature of G. max cv. HP-20-20.

Intra-subgeneric somatic hybridization has been investigated in order to establish reliable methods for the culture of heterokaryons and subsequent plant regeneration from somatic hybrid tissues of Glycine. The production of putative somatic hybrid callus from flow sorted G. argyrea G1626 x G. clandestina G1231 heterokaryons has already been published as a preliminary report (Jones et al., 1989). This callus subsequently became friable after transfer to agar-solidified SC2 medium (Hammatt et al., 1987). However, following regular subculture on the same medium, some calli have become compact, green and nodular. These tissues are being maintained to determine whether or not they will follow, under identical culture conditions, the same course of plant regeneration observed in morphologically similar tissues derived from unfused protoplasts of G. argyrea and G. clandestina (Hammatt et al., 1989a, 1988). While a high proportion of protoplast-derived tissues of G. argyrea (G1626) produce shoots, a much lower number from G. clandestina (G1231) undergo this process. The latter parent may be inhibiting shoot regeneration from the somatic hybrid tissues.

Heterokaryons produced by the fusion of hypocotyl protoplasts of G. max with those isolated from cotyledons of the perennial species of G. argyrea G1626 and G. clandestina G1826, the explants of which are highly regenerative, (Hammatt et al., 1989b; Kollipara and Hymowitz, 1989), have been collected using the flow cytometer. Callus from these experiments is also being cultured to determine its regenerative capacity for hybrid shoot production.

Conclusions: Protoplast-derived callus of G. max is, to date, recalcitrant to plant regeneration. Hence, somatic hybrid tissues derived from the fusion of protoplasts of G. max with wild relatives may have lower regeneration potential than protoplast-derived tissues of the perennial parent. Consequently, it is important to culture as many heterokaryons as possible following protoplast fusion. In this respect, flow cytometry is a convenient procedure for obtaining large Glycine heterokaryon populations, and this technique may prove valuable in facilitating gene flow between soybean and its wild relatives.

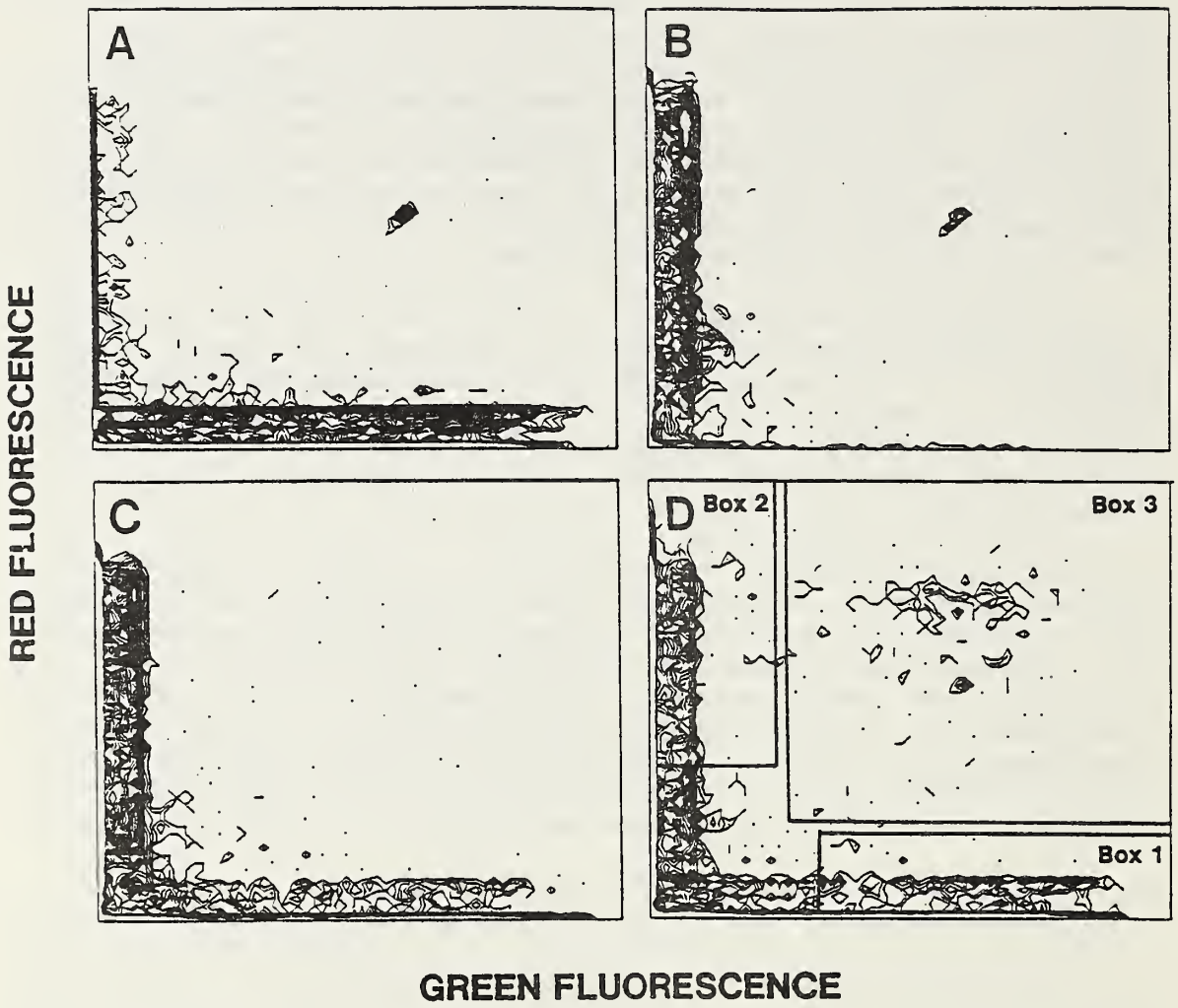

Figure 1. Graphs of green fluorescence plotted against red fluorescence for 20,000 protoplasts from (A) G. argyrea hypocotyls, (B) G. canescens cotyledons and a mixture of the two populations before (C) and after (D) fusion. In (D), there are 3564 unfused G. argyrea protoplasts (box 1), 6760 unfused G. canescens protoplasts (box 2) and 196 heterokaryons (box 3).

Table 1. Parameters relating to the sorting of Glycine heterokaryons by flow cytometry.

Parental Glycine species	Explant used as source of protoplasts	No. of heterokaryons recovered/ sorted	% heterokaryons in protoplast population	Response of heterokaryon derived tissues	Reference
G. max cv. HP-20-20 X G. canescens G1171	Hyp(DG) Cot(LG)	794^	94	SHS	Hammatt et al., 1988, 1989a
G. argyrea G1626 X G. clandestina G1231	Hyp(DG) Cot(LG)	366	95	PSHC	Jones et al. 1989
G. max cv. Essex X G. argyrea G1626	Hyp(DG) Cot(LG)	632	91	CC	---
G. max cv. Essex X G.clandestina G1826	Hyp(DG) Cot(LG)	718	95	CC	---

DG=dark-grown seedlings; LG=light-grown seedlings;
^ = estimated mean.
CC=cell colonies; PSHC=putative somatic hybrid callus; SHS=somatic hybrid shoots.

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2) Acrylamide concentration affects the resolution of leucine aminopeptidase isoenzymes from callus of soybean and Glycine canescens.

We have been interested, for a number of years, in the production of intersubgeneric somatic hybrid Glycine material. Early efforts concentrated on the chemical fusion of soybean crown gall protoplasts (see Pedersen et al., 1983) with those from cotyledons of G. canescens (Hammatt et al., 1987). Molecular markers have been sought that might assist in distinguishing the presence of both of the two parental genomes in putative somatic hybrid tissues. This note describes our results with the isoenzyme, leucine aminopeptidase (LAP) (E.C.3.4.11).

A crown gall line of G. max cv. 'Mandarin' was obtained from Dr. H. C. Pederson, Die Danske Sukkerfabrikker, Copenhagen, Denmark, and maintained by regular fortnightly transfer of 0.3-0.5 g of callus tissue to fresh hormone-free culture medium of Murashige and Skoog (1962). Axenic seedlings of G. canescens G1699 (CSIRO) were produced as described previously (Hammatt et al., 1987). Hypocotyl sections, 5 mm in length, from 7-day-old seedlings were incubated on 0.8% (w/v) agar-solidified medium of Uchimiya and Murashige (1974). The callus produced was subcultured to fresh medium every 2 weeks. Callus lines of G1699 and Mandarin were maintained in continuous light (1.6 Wm-2) at 27 C, either in 175-ml screw-capped glass jars containing 50 ml of culture medium, or in 9-cm diameter plastic Petri dishes each with 25 ml of medium.

Proteins were extracted 7 days after each subculture by grinding, using a pestle and mortar on ice, 1.0 g F.Wt. of callus in 1.0 ml of ice cold 50 mM Tris-HCl (pH 8) containing 200 gl^{-1} glycerol, 0.5 gl^{-1} dithiothreitol, 0.47 mgl^{-1} leupeptin and 0.68 mgl^{-1} antipain. The resulting slurry was centrifuged (11,500 x g for 10 min.), and the supernatant decanted and stored on ice until use within 1 hour of extraction.

Proteins were electrophoresed using an LKB Model 2001 vertical slab gel kit. The separation gels, 25 ml in volume, consisted of either 60 or 90 gl^{-1} (6.0% or 9.0% w/v) acrylamide and 1.6 or 2.4 gl^{-1}, respectively, of N,N' methylene bisacrylamide, and 100 ml^{-1} glycerol dissolved in 75 mM Tris-HCl (pH 8.4). The stacking gel consisted of 30 gl^{-1} acylamide, 0.8 gl^{-1} bisacrylamide and 100 $ml l^{-1}$ glycerol in a 17 mM Tris-HCl buffer (pH 6.8). Before pouring, each gel was degassed under vacuum and the polymerants N,N,N',N' tetramethylene ethylene diamine (TEMED) and ammonium persulphate were added to give final concentrations of 1 $ml l^{-1}$ and 0.2 gl^{-1}, respectively. Ten wells, 200 ul in volume, were created in each stacking gel using a plastic comb. Two ul of 1 gl^{-1} bromophenol blue was added to each well, followed by 100 ul of protein sample.

Proteins were electrophesed, using 10 mA constant current and a running buffer of 14.2 gl^{-1} glycine and 3.0 gl^{-1} Tris (pH 8.3), until the tracking dye had run from the gel (3-5 hours). Each gel was stained for LAP activity by incubation, in the dark, in 50 ml of a 0.2 M Tris maleate buffer (pH 6.0) containing 15 mg of L-leucyl-B-naphthylamide HCl dissolved in 30 ml of 50% (v/v) acetone, and 50 mg of Fast Black K salt. Reactions were stopped by washing the gels in tap water. The background staining was reduced by two or three 10-20 min. passages through acetic ethanol (75% v/v ethanol : 10% v/v glacial acetic acid) before fixation in 10% acetic acid.

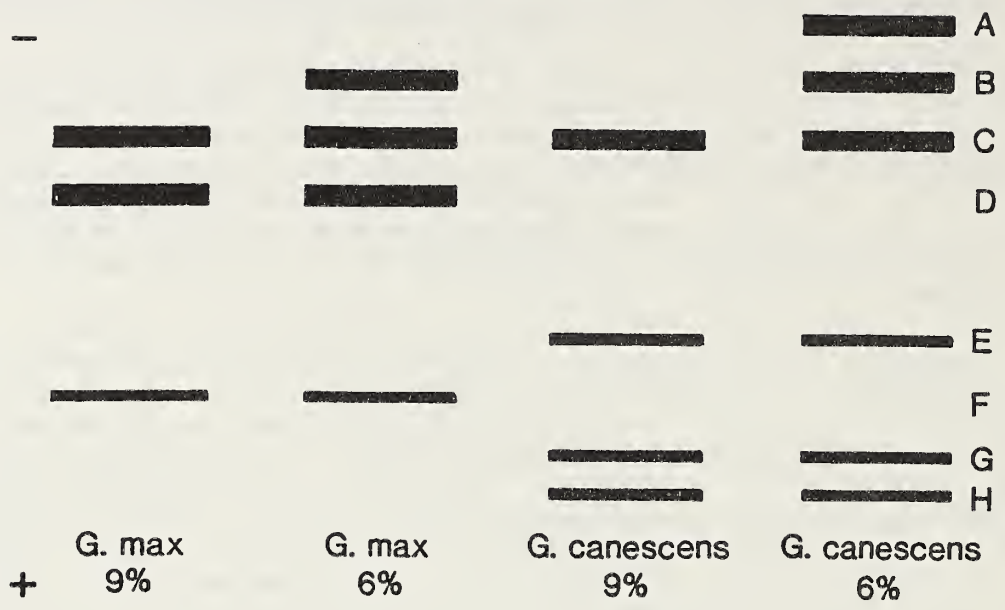

Figure 1. Zymograms of leucine amino peptidase tissue of soybean and G. canescens, obtained using gels containing 6.0% and 9.0% (w/v) acrylamide.

As shown in Figure 1, LAP isozymes separated in 9.0% acrylamide to give a protein, C, common to both parents. This band usually showed poor definition. Bands D and F were specific to soybean, while bands E, G, and H were found only in extracts from G. canescens. Clearly, with so many parent-specific bands for both species, LAP would be an important isozyme to investigate in putative somatic hybrid tissues.

When the same protein extracts were run through a 6.0% acrylamide gel, all of the proteins moved faster, and parent-specific isozymes D-H remained in the same relative positions. For soybean, separation of band C into two separate bands, C and the new protein, B, was achieved. For G. canescens, protein C separated into 3 bands, namely, C, B, and A. The latter (A) was a protein found only in G. canescens and could constitute another marker for detecting the genome of G. canescens in hybrid tissues.

These observations emphasize the importance of investigating acrylamide concentration as an important factor involved in determining optimum definition of all Glycine LAP isozymes during PAGE. Further work is required to investigate other isozymes as markers for hybridity and the effect of acrylamide concentration upon band resolution in each zymogram.

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1) Origin of new dwarf mutation.

Six independent sources for dwarf mutations, representing five loci, are maintained in the Soybean Genetic Type Collection. The strains T210 and T243 carry the df2 mutation, T244-df3 locus, T256-df4 and T262-df5 locus, respectively (Palmer and Kilen, 1987).

In the F3 progeny row, from the cross of T258H (St4st4) by BDII (Bill Davis II sterile), which was observed for sterility, short plants with dark green, crinkled leaves were found. In this progeny row, 197 plants had normal phenotype, and 14 plants exhibited dwarfness. Only 12 seed were harvested from dwarf plants.

Twenty-six normal plants were harvested from the F3 progeny; consequently, F4 progeny rows were observed for plant growth segregation. Thirteen F4 progenies were homozygous for plant height, and 13 progenies segregated for normal and dwarf plants. Results on segregation are presented in Table 1. Sporadically, miniature (up to 5 cm) very dark green plants were observed in segregating progenies, suggesting an involvement of additional loci.

Table 1. Segregation for dwarf mutation in F4 progenies of soybean.

	Phenotype		Ratio
Progeny	Normal	Dwarf	Normal/Dwarf
C-175	37	7	5.3
C-180	64	10	6.4
C-184	39	8	4.9
C-184	27	4	6.7
C-186	13	1	13.0
C-189	71	8	8.9
C-191	88	9	9.8
C-192	69	8	8.6
C-196	41	10	4.1
C-197	63	6	9.0
C-198	103	13	7.9
C-199	38	10	3.8
C-200	105	16	6.6

Seeds harvested from dwarf plants were planted in the greenhouse. The dwarf character was temperature-sensitive. Under a 28 C temperature regime, dwarf plants grew to 62.97 cm, whereas in the field, dwarf plants were only 15-25 cm tall. Dwarf plants produced fertile pollen, but set only a few individual pods. This dwarf mutation seems to have a pleiotropic effect on plant fertility, and seems to be involved in several biochemical pathways. Genetic and developmental studies of this dwarf mutation are underway.

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1) Origin of the w4-m allele.

Plants of the "w4-mutable" line of soybean are chimeral for anthocyanin pigmentation (Palmer et al., 1989). Somatic and genetic analyses have established that the mutable trait is conditioned by an unstable recessive ("mutable") allele of the w4 locus that conditions anthocyanin biosynthesis (Groose et al., 1988; Groose and Palmer, 1990; Groose et al., 1990). The gene symbol w4-m has been assigned to the mutable allele. Allele w4-m was derived from a stable wild-type W4 progenitor allele and reverts at high frequency to a stable wild-type W4 allele. This communication outlines the origin of the w4-mutable line and the w4-m allele.

The w4-mutable line consists of the self-progenies of four F_6 plants derived from a cross between two experimental breeding lines. The parents, "X1878" and "X2717", breed true for white and purple flowers, respectively. Progeny of the cross were advanced to the F_7 without regard to flower color. Progenies were advanced in bulk to the F_4 when single-plant selections were made. At the F_5, a single progeny row was selected and bulk threshed to produce an F_6 population from which 60 single plants were selected and threshed separately to produce 60 F_7 progenies. In July 1983 at the Asgrow Seed Company nursery at Stonington, Illinois, it was observed that four of the 60 F_7 progenies were segregating for mutable-flowered and purple-flowered plants. These four sublines and their progenies constitute the w4-mutable line. The other 56 F_7 progenies bred true for purple flowers.

Sublines of the w4-mutable line are identified by pedigree where the first numeral designates an F_6 ancestor. For example, most genetic analyses performed by Groose and Palmer (1990) and Groose et al. (1990) were performed with highly mutable F_{10} plants of subline "W4M-3-1-12-1" where "W4M" designates "w4-mutable" and "3", "1", "12", and "1" identify the F_6, F_7, F_8, and F_9 parentage, respectively.

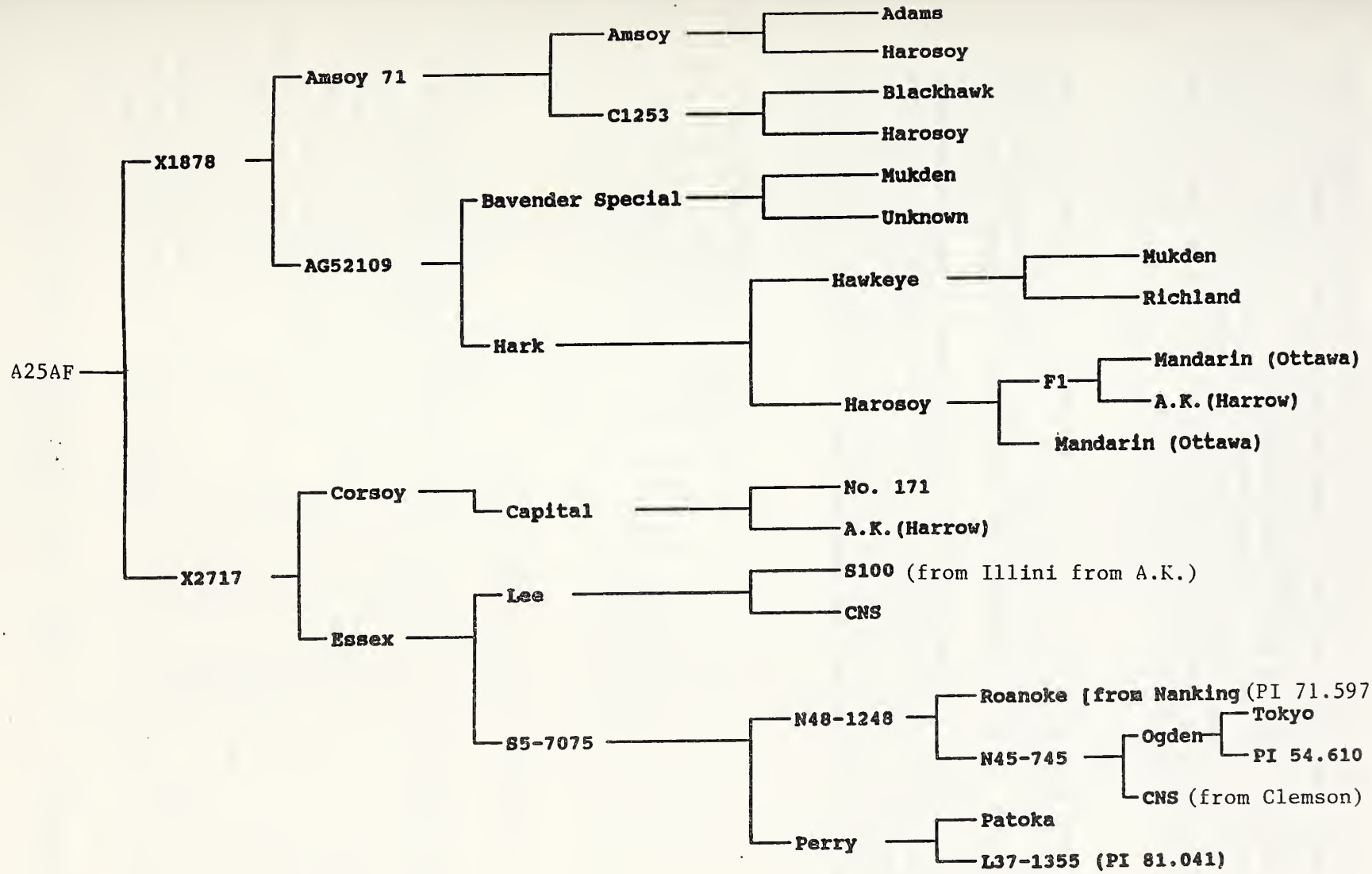
Several lines of evidence indicate that the unstable recessive w4-m allele arose from a stable wild-type (W4) progenitor. Both parents of the w4-mutable line breed true for wild-type dominant alleles of the w4 locus. Parent X2717 breeds true for purple flowers, indicating a W1W1,W4W4 genotype at the major loci conditioning anthocyanin pigmentation. Parent X1878 breeds true for white flowers, indicating homozygosity for recessive alleles at the w1 locus. Results of genetic analysis of X1878 indicated homozygosity for wild-type dominant alleles at the w4 locus in X1878 (Table 1) where the genetic analysis was performed using purple-flowered Harosoy, and the white-flowered and near-white-flowered Harosoy isolines (designated "Harosoy-w1" and "Harosoy-w4", respectively) obtained from the Soybean Genetics Collection, Dr R.L. Bernard, Curator, Dept. of Agronomy, Univ. of Illinois, Urbana IL 61801. Segregation of purple- and white-flowered plants (but no near-white- or mutable-flowered plants) in F_3 and F_4 progenies from remnant seed of the original cross of X2717 X X1878 confirmed the assignment of W1W1,W4W4 and w1w1,W4W4 genotypes to X2717 and X1878, respectively (data not presented).

An analysis of the origin the w4-mutable line (outlined above) suggests that the mutation from W4 to w4-m occurred in the F_4 or F_5 generation. Of the

Table 1. Genetic analysis of white-flowered Asgrow experimental line X1878

Tester		F_1		F_2 Segregation				
				No. seedlings				
Isoline	Genotype	No.	Phenotype	WT	GR	$X^2_{1df}(3:1)$	$X^2_{1df}(9:7)$	Probability
Harosoy	W1W1,W4W4	3	WT [∫]	208	65	0.207		0.500<P<0.750
Harosoy-w1	w1w1,W4W4	8	WH	0	269			
Harosoy-w4	W1W1,w4w4	16	WT	150	122		0.134	0.500<P<0.750

[∫] WT=wild type; WH=white; GR=green, as defined in Table 1 of the following article (Groose et al., 1990).



[L37-1355 was a rogue in PI 81.041 and may or may not be genetically derived from PI 81.041]

Figure 1. Pedigree of X25AF

60 F_7 lines, 56 lines were true-breeding wild-type, indicating a W4W4 genotype in the F_6; two lines (W4M-1 and W4M-4) were segregating approximately 3 wild-type : 1 mutable, indicating a W4w4-m genotype in the F_6; and two lines (W4M-2 and W4M-3) were segregating approximately 1 wild-type : 9 mutable, indicating a w4-mw4-m genotype in the F_6. The non-Mendelian F_6 genotypic ratio (56 W4W4 : 2 W4w4-m : 2 w4-mw4-m) indicates that the mutation from W4 to w4-m occurred in a sector of the germ line of the single F_4 plant (or one of its F_5 progeny) from which the w4-mutable sublines are descended.

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2) Genetic analysis of the w4-mutable line.

Plants of the "w4-mutable" line of soybean are chimeral for anthocyanin pigmentation (Palmer et al., 1989). Mutable plants produce both near-white and purple flowers, as well as flowers of mutable phenotype with purple sectors on near-white petals. Somatic and genetic analyses of the w4-mutable line indicate that the mutable trait is conditioned by an unstable recessive ("mutable") allele of the w4 locus that conditions anthocyanin biosynthesis (Groose et al., 1988; Groose et al., 1990). This communication presents results of additional genetic analyses that support the assignment of the gene symbol "w4-m" to the mutable allele. The pleiotropic effects of the major loci w1 and w4 on anthocyanin pigmentation of flowers and hypocotyls of plants used in this study are summarized in Table 1. The symbols for phenotype, presented in Table 1 (WT, MU, NW, WH, and GR) are used in subsequent tables. 'Harosoy' isolines used in hybridization experiments were obtained from the Soybean Genetics Collection, Dr R.L. Bernard, Curator, Dept. of Agronomy, Univ. of Illinois, Urbana IL 61801.

F_3 generation data from the cross of the highly mutable subline

Table 1. Anthocyanin pigmentation as conditioned by the w1 and w4 loci in soybean

Phenotype	Pigmentation: Flower	Pigmentation: Hypocotyl	Genotype[a]
WT = wild-type	purple	purple	W1--,W4--
NW = near-white	near-white	GR = green	W1--,w4w4
MU = mutable	mutable[b]	mutable[c]	W1--,w4-mw4-m or W1--,w4-mw4
WH = white	white	green	w1w1,----

[a] Genotypes of mutable plants are as determined by genetic analysis in the present study.

[b] Flowers produced by mutable plants include flowers with purple sectors on a near-white background, as well as several periclinal chimeral types (illustrated in Groose et al. 1988) that result from somatic reversion in different cell layers.

[c] Hypocotyls of mutable seedlings exhibit purple flecks and stripes on a green background.

Table 2. Segregation in F_3 families derived from cross between M4-mutable line and wild-type Harosoy line.

Cross no.	No. F_3 families[a]: true-breeding WT	segregating 3WT:1MU[b]	segregating 1WT:9MU[b]	$X^2_{2df}(1:2:1)$	Probability
1	3	12	6	1.285	0.500<P<0.750
2	7	12	3	1.636	0.250<P<0.500
3	5	10	8	1.174	0.500<P<0.750
4	6	11	6	0.044	0.975<P<0.990
5	6	9	10	3.240	0.100<P<0.250
6	5	16	1	6.000	0.025<P<0.050
7	5	13	5	0.392	0.900<P<0.950
8	17	0	0	51.000	P<0.005
9	5	12	3	1.200	0.500<P<0.750
10	5	13	3	1.571	0.250<P<0.500
Total	64	108	45		
		X^2_{2df}pooled = 3.331			0.100<P<0.250
		X^2_{18df}homogeneity = 64.211			P<0.005

Table 3. Segregation in F_3 progenies of mutable F_2 plants descended from cross between w4-mutable line and wild-type Harosoy line

Cross no.	Family no.	F_3 Segregation[a] WT	MU	X^2_{1df}(11:89)	Probability
6	1	1	39[b]	3.102	0.050<P<0.100
7	1	5	51	0.319	0.500<P<0.750
"	2	0	28	3.575	0.050<P<0.100
"	3	1	41[b]	3.350	0.050<P<0.100
"	4	2	49[b]	2.788	0.050<P<0.100
"	5	0	19[b]	2.424	0.100<P<0.250
9	1	4	41	0.267	0.500<P<0.750
"	2	21	24	55.907	P<0.005
"	3	7	47[b]	0.143	0.500<P<0.750
10	1	4	44[b]	0.430	0.500<P<0.750
"	2	3	27[b]	0.053	0.750<P<0.900
"	3	10	44[b]	2.774	0.050<P<0.100
Total		58	454		
(%)		(11.3)	(88.7)		
		X^2_{11df}homogeneity = 75.132			P<0.005

[a] Evaluation of anthocyanin pigmentation of seedling hypocotyls
[b] Includes a small number of seedlings (<10%) of GR phenotype

Table 4. Genetic analyses of crosses between w4-mutable and wild-type lines.

Wild-type line	F_1		F_2 Segregation[a]			
			No. seedlings			
	No.	Phenotype	WT	MU[b]	X^2_{1df}(3:1)	Probability
PI 79593[c]	6	WT	179	67	0.656	0.250<P<0.500
PI 407293[d]	8	WT	189	48	2.848	0.050<P<0.100
Minsoy	4	WT	319	50	25.800	P<0.005
AM revertant[e]	49	WT	697	248	0.769	0.250<P<0.500

[a] F_2 is a composite of seed from all F_1 individuals. Evaluation of anthocyanin pigmentation of seedling hypocotyls.
[b] Any seedlings of GR phenotype were included in the MU class
[c] G. gracilis
[d] G. soja
[e] Plants of two true-breeding wild-type F9 sublines (AM-2-9-6 and AM-3-6-4) of w4-mutable

Table 5. Segregation in F_3 progenies of mutable F_2 plants descended from cross between w4-mutable line and nearwhite-flowered Harosoy-w4 isoline

Cross no.	No. F_3 families: "True-breeding" WT or MU: True-breeding WT	Segregating 3WT:1MU[a]	Segregating 1WT:9MU[b]	Total	Segregating 3WTorMU:1GR: Segregating 3WT:1GR	Segregating 3MU&WT:1GR	Total	True-breeding GR	$X^2_{2df}(1:2:1)$	Probability
1			7	7		10	10	5	0.545	0.750<P<0.900
2			4	4	1	12	13	6	0.740	0.250<P<0.500
3		2		2	1	10	11	7	2.700	0.250<P<0.500
4			1	1		10	10	9	6.400	0.025<P<0.050
5				0	3	8	11	6	5.706	0.050<P<0.100
6			8	8		9	9	4	1.952	0.250<P<0.500
7	1	1	5	7	1	9	10	3	1.600	0.250<P<0.500
8			5	5		11	11	4	0.300	0.750<P<0.900
9			5	5	1	9	10	6	0.143	0.900<P<0.950
10	1		6	7		9	9	5	0.809	0.500<P<0.750
	--	--	--	--	--	--	---	--		
Total	2	3	41	46	7	97	100	55		
									X^2_{2df} pooled = 0.834	0.500<P<0.750
									X^2_{18df} homogeneity = 20.061	0.250<P<0.500

[a] Within some families, a small number of seedlings of GR phenotype are included in the MU class

[b] Within these families, the dominant class included approximately 90% mutable and 10% wild-type seedlings

Table 5. Segregation in F_3 progenies of mutable F_2 plants descended from cross between w4-mutable line and nearwhite-flowered Harosoy-w4 isoline

Cross no.	No. F_3 families: "True-breeding" WT or MU — True-breeding WT	Segregating 3WT:1MU[a]	Segregating 1WT:9MU[b]	Total	Segregating 3WTorMU:1GR — Segregating 3WT:1GR	Segregating 3MU&WT:1GR	Total	True-breeding GR	X^2_{2df}(1:2:1)	Probability
1			7	7		10	10	5	0.545	0.750<P<0.900
2			4	4	1	12	13	6	0.740	0.250<P<0.500
3		2		2	1	10	11	7	2.700	0.250<P<0.500
4			1	1		10	10	9	6.400	0.025<P<0.050
5				0	3	8	11	6	5.706	0.050<P<0.100
6			8	8		9	9	4	1.952	0.250<P<0.500
7	1	1	5	7	1	9	10	3	1.600	0.250<P<0.500
8			5	5		11	11	4	0.300	0.750<P<0.900
9			5	5	1	9	10	6	0.143	0.900<P<0.950
10	1		6	7		9	9	5	0.809	0.500<P<0.750
	--	--	--	--	--	--	---	--		
Total	2	3	41	46	7	97	100	55		

X^2_{2df} pooled = 0.834 0.500<P<0.750

X^2_{18df} homogeneity = 20.061 0.250<P<0.500

[a] Within some families, a small number of seedlings of GR phenotype are included in the MU class

[b] Within these families, the dominant class included approximately 90% mutable and 10% wild-type seedlings

W4M-3-1-12-1 with purple-flowered Harosoy (genotype W1W1,W4W4) provided confirming evidence that mutability is conditioned by a single unstable recessive allele (Table 2). Approximately 20 F_3 families were derived from F_2 plants from remnant seed of each of the F_2 families of Table 2 of Groose et al. (1990). The phenotype of each F_2 plant was evaluated at flowering. All wild-type F_2 plants produced F_3 progenies that either bred true for wild type or segregated approximately 3 wild-type : 1 mutable. All mutable F_2 plants produced mostly mutable F_3 progeny. In these F_3 families, the proportion of wild-type segregates ranged from 0% to nearly 50%, with most families segregating approximately 1 wild-type : 9 mutable. This non-Mendelian segregation is most simply explained by the reversion of mutable alleles in the germ line of mutable F_2 plants. Segregation of progeny in 12 F_3 families derived from mutable F_2 plants are presented in Table 3.

For eight of the ten F_2 families derived from the w4-mutable X Harosoy cross, the F_2 genotypic ratio was a close fit to the expected 1 : 2 : 1 ratio (Table 2), where the three classes are presumed to be derived from F_2 plants that were, respectively, homozygous for wild-type alleles, heterozygous for wild-type and mutable alleles, and homozygous for mutable alleles. The non-Mendelian proportion (approximately 10%) of wild-type plants that segregated in the latter class of F_3 family may be attributed to reversion of the mutable allele in the germ line of mutable F_2 plants. For two of the ten F_2 families, families no.6 and no.8, the F_2 genotypic ratio was a poor fit to the expected 1 : 2 : 1 ratio. These are F_2 families in which the fit to the expected 3 : 1 F_2 phenotypic ratio was poor (Table 2 of Groose et al, 1990). Possible explanations for these deviations (and for the lack of homogeneity among families) based on germ-line reversion early in development of the F_1 (for family no.6) and germinal reversion in the mutable parent of the F_1 (for family no.8) were discussed by Groose et al. (1990). Family no.3, which deviated from the expected 3 : 1 phenotypic ratio in the F_2 (Table 2 in Groose et al., 1990), did not deviate from the expected 1 : 2 : 1 genotypic ratio as based on the F_3 data (Table 2). This may be the result of chance statistical variation in one of the experiments.

Mutable plants were crossed to several other wild-type lines that ranged from distantly related (including wild relatives of soybean) to very closely related lines (wild-type sublines derived from w4-mutable). The highly mutable F_{10} subline W4M-3-1-12-1 was crossed with Harosoy and plant introductions PI 79593 (G. gracilis Skvortz.) and PI 407293 (G. soja Sieb. and Zucc.). Moderately mutable F_9 sublines, W4M-2-2-1 and W4M-2-8-1, were crossed with PI 27890 "Minsoy". F_9 sublines of low mutability (W4M-2-14-3, W4M-2-16-3, W4M-3-1-4, W4M-3-11-3, and W4M-3-14-2) were crossed with two true-breeding wild-type F_9 sublines (W4M-2-9-6 and W4M-3-6-4) that descended from mutable F_7 plants. F_1 and F_2 generation data from these crosses also indicate that mutability is conditioned by an unstable recessive allele of a single locus (Table 4). All F_1 progeny were wild type. Three of the four F_2 segregations were approximately 3 wild-type : 1 mutable. The F_2 segregation of the cross between w4-mutable and Minsoy produced an excess of wild-type segregates, perhaps as a result of reversion early in the development of the germ line of these F_1 plants. (Note: None of the w4-mutable X Minsoy F_1 plants was a germinal revertant, as indicated by segregation within all four F_2 progenies; R.W. Groose, unpublished results.) It is also possible that the

mutable allele is especially unstable in the w4-mutable X Minsoy background.

F_3 generation data from the cross of w4-mutable with Harosoy-w4 (genotype W1W1,w4w4) confirmed that mutability is conditioned by an unstable recessive allele of the w4 locus (Table 5). Approximately 20 F_3 families were derived from F_2 plants from remnant seed of F_2 families no.1 through no.10 in Table 3 of Groose et al. (1990), all of which had been derived from mutable F_1 plants. Germinal reversion of mutable alleles in F_1 and F_2 plants, together with segregation of unstable alleles, revertant W4 alleles and recessive w4 alleles resulted in six types of F_3 family as based on evaluation of seedling hypocotyls. The six types of F_3 family could be classified into those that were (i) "true-breeding" for mutability or wild type; (ii) segregating 3 mutable (or wild-type) : 1 green; and (iii) true-breeding green. These three classes of F_3 family closely fit a 1 : 2 : 1 genotypic ratio where the three classes represent F_2 individuals that were (i) "homozygous" for mutable alleles or their revertant W4 derivatives; (ii) heterozygous for mutable (or revertant) and recessive w4 alleles; and (iii) homozygous for recessive w4 alleles.

F_1 and F_2 generation data from a cross between the highly mutable w4-mutable subline W4M-3-1-12-1 and Harosoy-w1 (genotype w1w1,W4W4) indicate nonallelism of the mutable trait to the w1 locus (Table 6). All F_1 plants were wild type. F_2 families segregated 9 wild-type : 3 mutable : 4 white (as based on evaluation of flowering plants) and 9 wild-type : 3 mutable : 4 green (as based on evaluation of seedling hypocotyls). The close fit to the expected dihybrid F_2 ratios and the homogeneity among F_2 families indicates that none of these six F_1s was germinally revertant.

The data of Tables 5 and 6 provide compelling evidence that allelism is at the w4 locus, not the w1 locus, for mutability conditioned by a single unstable recessive allele which is designated w4-m.

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3) Genetics and linkage studies of a new chlorophyll-deficient mutant.

We reported F2 inheritance data of the v2 locus (T312), and gave examples of segregation distortion (Honeycutt et al., 1988). In crosses of cultivar Evans x T312 (v2 v2) we had six F1 plants. In four of the six F1 plants, the F2 ratio of green : chimera plants was 3.5:1. Two of the six F1 plants segregated for yellow lethal seedlings, in addition to green plants and chimeric plants. Three green plants and three chimeric plants were saved from one of these F2 progenies that contained all three phenotypes.

In the F3, two of the green plants segregated for v2 and the other green plant segregated for the yellow lethal (A88-612). Two of the chimeric plants (A88-607 and A88-609) segregated for the yellow lethal and the other chimeric plant bred true.

Twenty F3 plants from family A88-612 were single-plant threshed. F4 progenies were evaluated in the sandbench. Six families were nonsegregating and 14 families segregated about 3 green : 1 yellow lethal (Table 1). A total of 38 F3 plants from families A88-607 and A88-609 were single-plant threshed. Progenies were evaluated in the sandbench. The ratio of nonsegregating to segregating families was 12:26. Within segregating families the ratio of green : yellow lethal was about 3:1 (Table 1).

Honeycutt et al., (1990) reported that v2 was located on trisomic A. Our objective was to test whether or not the yellow lethal mutant was a new allele at v2 by determining if the new mutant was located on trisomic A.

Green plants from family A88-612 were selected to use in crosses with trisomic A. Individual plants were maintained and progeny-tested to identify heterozygotes. Crosses were made in the greenhouse during spring 1989. Only F1 seed from crosses involving known heterozygotes were checked for chromosome number. The F1 seedlings were transplanted to the field. Each 40- and 41-chromosome F1 plant was threshed individually. Progenies were evaluated in the sandbench. The data show that the yellow seedling lethal is not on trisomic A (Table 2).

In summary, the mutant is inherited as a single-gene recessive and the mutant is not located on trisomic A. Therefore, we have isolated a new spontaneously occurring chlorophyll-deficient mutant from a F2 population derived from a cross of Evans x T312.

Table 1. F4 data from a cross between Evans and v2 v2. This cross also segregated for a new chlorophyll-deficient mutant.

	Number of F4 plants				
Family	Green	Chimera	Yellow	X^2 (3:1)	P
A88-612	721		232	0.22	0.70>P>0.50
A88-607		515	181	0.38	0.70>P>0.50
A88-609		709	250	0.58	0.70>P>0.50

Table 2. Test of linkage of a chlorophyll-deficient mutant and trisomic A.

Chromosome number	Number of F1 plants	Number of F2 plants Green	Yellow	X^2 (3:1)	P
40	8	3278	1066	0.49	0.50>P>0.30
41	2	587	185	0.44	0.70>P>0.50

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R. G. Palmer - USDA

4) Linkage studies with an unknown trisomic that occurred spontaneously in ms1 ms1 soybean plants.

Introduction: The major sources of aneuploids in soybean are synaptic mutants. Two trisomics, Tri A and Tri B, were isolated from progeny of asynaptic mutant T241 (st2) (Palmer, 1974). Two other trisomics, Tri C and Tri D, arose from the progeny of desynaptic mutant T258 (st4) (Palmer and Heer, 1976). The first example of trisomic inheritance in soybean was the location of the v2 chimera gene to trisomic A (Newhouse et al., 1983). Sadanaga and Grindeland (1984) were able to locate the flower color locus w1 on the satellite chromosome by using a trisomic line (Tri S) derived from irradiated soybeans. The development of protein electrophoresis and molecular techniques have provided researchers with numerous genetic markers that usually have

codominant alleles. An isozyme marker, Dial (diaphorase) locus, was located to the Tri D chromosome by using starch gel electrophoresis (Hedges, 1989).

The objective of our research was to test for linkage between the unknown trisomics found in msl msl plants in 1986 with v2 (Tri A), wl (Tri S) and Dial (Tri D). The modified ratios in F2 generation would indicate trisomic inheritance.

Materials and methods: A male-sterile, purple-flowered, 42-chromosome plant (mslmslWlWlDialDialV2V2) was found in the progeny of a mslmsl plant in the greenhouse in the summer of 1986. Crosses have been made between this 42-chromosome plant and white flower, fertile plants (cv. 'Wye', MslMslwlwldialdialV2V2). Nine F_2 seeds were obtained from those crosses. Four of the F_1 plants were trisomic, i.e., FA1-2, FA1-4, FA1-7, and FA1-8; the other five F_1 plants were disomic. The F2 seeds (FA88- line) from both 40- and 41-chromosome F1 plants were harvested separately and placed on germination paper. Samples for isozyme examination were taken from a cotyledon from each seedling (Cardy and Beversdorf, 1984a and b) to analyze diaphorase pattern. Chromosome number was determined (Palmer and Heer, 1973), and seedlings transplanted to pots.

In order to increase the number of samples of the unknown trisomic with v2 (Tri A and for isozyme examination, 41-chromosome plants with dialdialV2V2 alleles selected from FA88- lines were crossed with Genetic Type T312 (DialDialv2v2). Chromosome numbers of F_1 plants were checked. F_2 seeds from the 40- and 41-chromosome F_1 plants were harvested separately. The segregation tests for v2 and dial were conducted with the F_2 population.

Results and discussion: The segregation ratios for flower color genes wl, chimeric gene v2, and diaphorase pattern Dial were analyzed in the 40- and 41-chromosome progenies (Table 1). The results indicated that there was no linkage between the unkown trisomic and wl and Dial. Therefore, the extra chromosome of the unknown trisomic is different from the extra chromosome in Tri S and Tri D.

The segregation ratios for v2 in both 40- and 41-chromosome progenies were significantly different from 3:1 ($P<0.10$ and $P<0.001$, respectively). According to previous study on the v2 allele (Honeycutt et al., 1988), the F_2 segregation ratios of most crosses with the v2 mutant deviated from the expected 3:1 ratio. The average ratio for each cross was from 3.5:1 to 10.5:1 in their results. Our results confirmed their observations. The segregation ratios were 5.5:1 for progeny from 40-chromosome plants and 9.9:1 for that from 41-chromosome plants (Table 1). There is, therefore, no evidence to indicate that the v2 allele is located on the unknown trisomic.

The unknown trisomics originated from a 42-chromosome plant that was obtained from mslmsl plants. This 42-chromosome plant may be a double trisomic (2n+1+1) that contained two different extra chromosomes, or a tetrasomic (2n+2) that contained two identical extra chromosomes. In the F_1 progeny from crossing of this 42-chromosome plant with disomic plants, only 40- and 41-chromosome plants were obtained. To determine whether the extra chromosomes from different individuals are the same or diferent will require additional research.

Table 1. Segregation patterns of wl, v2, and Dial in 40- and 41-chromosome progenies.

	Flower color		Chimera		Diaphorase*		
	Wl	wlwl	V2	v2v2	A	H	B
41-chromosome progeny	83	30	158	16	73	124	66
	X^2 (3:1) 0.15		X^2 (3:1) 22.47		X^2 (1:2:1) 1.23		
	0.80>P>0.70		P<0.001		0.70>P>0.50		
40-chromosome progeny	222	75	66	12	49	96	43
	X^2 (3:1) 0.01		X^2 (3:1) 3.82		X^2 (1:2:1) 0.47		
	0.95>P>0.90		0.10>P>0.05		0.80>P>0.70		

* A=DialDial; H=Dialdial; B=dialdial.

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1) Evaluation of specialty soybean germplasm for corn earworm resistance.

The increasing awareness of the disadvantageous effects of cholesterol in dietary meals has raised the demand for food having low or no cholesterol content. The effect of such demand has generated not only an increase in supply of soybean but simultaneously expanded the use of the plant product from mere animal feed to an ingredient widely used in technology, industry and for human consumption (Scott et al., 1970). The expanding diversity of soybean utilization has created tremendous interest for the breeding of cultivars ideally suited for each use.

Among the major impediments to profitability and the realization of yield increase is insect pest damage. Corn earworm (Heliothis zea-Boddie) known as a major soybean pest can cause serious economic losses from damage done to foliage or pods (Hill, 1987). If control measures are not taken in time, pod feeding could go undetected, resulting in severe crop loss. Small pods attacked by this pest either drop from the plant or fail to develop properly (Scott et al., 1970). Developing seeds are eaten in their pods, thus reducing yield. According to Boldt et al., (1975) and Smith and Bass (1972) each corn earworm larvae can destroy up to eight pods. If the population of larvae increases during the early developmental stage of the beans, then chemical treatment may become necessary in order to save the crop. However, the increased use, and higher cost of chemical, compounded with its environmental hazards, have caused severe concerns not only among farmers and environmentalists but also among soybean consumers. Such concerns have left plant breeders and other plant research scientists no alternative other than to develop cultivars resistant to pests capable of causing economic loss. It is therefore the objective of this study to identify specialty soybean germplasm that is resistant to corn earworm pod damage.

Materials and methods: Fifty-one specialty soybean cultivars belonging to maturity groups (MG) III, IV, and V were planted June 6, 1989. Each cultivar was planted in a 4.5-meter single row plot with row spacing of 75 cm. There were four replications arranged in randomized complete block design. Traflon was incorporated at a rate of 1.1 pint per acre prior to planting to control weed growth. Plants were harvested at ground level between mid October and December, 1988. Five plants were randomly selected from each row. The number of damaged pods was recorded and percentage of damaged pods per plant was calculated.

Experimental results: Percent pod damage by corn earworm for each cultivar according to maturity groups are presented in Tables 1, 2, and 3.

The percentage of pods damaged by corn earworm in maturity groups III, IV, and V ranged from 0.17 to 1.25, 005 to 1.75, and 0.08 to 1.03, respectively. The most resistant cultivar in maturity group III was 'Fuji', while 'Pella' was most susceptible (Table 1).

Pod damage resistance among cultivars in maturity groups IV and V showed 'Sooty' and 'Sanga' of MG V and PI 417,288 of MG V to be highly resistant,

while 'Kaikoo' of MG IV and PI 417,052 of MG V as susceptible cultivars (Tables 2 and 3).

Results from two years' studies on maturity groups III and IV revealed that, among the cultivars in MG III, Fuji was highly resistant against corn earworm and 'Williams 79' was most susceptible. For MG IV, Sanga was most resistant, while 'M. Summer' was most susceptible (Table 1 and 2, respectively). Owing to extremely poor or no germination, there are no data for cultivars 'Jogun' and 'Imperial' of MG IV.

Results from these preliminary data indicate that Fuji, Sanga, and PI 417,288 might be good source stock for transferring the corn earworm resistance genes to new specialty soybean cultivars.

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Table 1. Pod damage evaluation and comparison of 1988 and 1989 pod damage data MG III soybean cultivars.

Cultivar	% pod damage/plant 1988	1989	$\bar{X}$
Wolverine	1.74	0.85	1.30
Willomi	1.19	0.69	0.94
Fuji	1.32	0.17	0.75
Oakland	3.84	0.22	2.03
Guelph	1.69	0.37	1.03
Columbia	4.47	0.62	2.55
Kura	2.36	1.25	1.81
Kim	0.95	0.73	0.84
Williams-79	4.37	1.13	2.75
Pella	2.61	1.33	1.97
Kanrich	2.99	1.23	2.11

while 'Kaikoo' of MG IV and PI 417,052 of MG V as susceptible cultivars (Tables 2 and 3).

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Kim	0.95	0.73	0.84
Williams-79	4.37	1.13	2.75
Pella	2.61	1.33	1.97
Kanrich	2.99	1.23	2.11

Table 2. Pod damage evaluation and comparison of 1988 and 1989 pod damage data for MG IV cultivars.

Cultivar	% pod damage/plant 1988	1989	X
PI 85,505	1.08	0.27	0.68
Emperor	1.97	0.37	1.17
Kahala	4.00	1.38	2.69
Funk Delicious	2.05	0.45	2.50
M. Summer	10.29	0.78	5.54
Verde	4.41	0.87	2.64
Hahto/Michigan	--	1.59	1.59
Sato	2.25	0.46	1.36
Peking	1.39	0.32	0.86
Shiro	1.99	0.42	1.21
Jefferson	--	0.82	0.82
Aoda	3.61	0.65	2.13
Ware	4.39	0.94	2.67
Jogun	--	--	--
Wilson 5	2.60	0.23	1.42
Kailu	4.31	1.01	2.66
PI 82,264	4.30	0.36	2.33
Kaikoo	2.92	1.75	2.34
PI 339,984	1.51	0.82	1.67
Imperial	--	--	--
Sanga	0.67	0.09	0.38
Emerald	3.67	0.65	2.21
Kingston	2.31	0.28	1.80
Norredo	2.31	0.28	1.32
Sooty	5.65	0.05	2/85

Table 3. Pod damage evaluation for MG V cultivars.

Cultivar	% pod damage
PI 417,159	0.55
PI 423,827	0.65
PI 416,771	0.20
PI 417,052	1.03
PI 408,155	0.35
PI 423,758	0.43
PI 423,759	0.36
PI 416,467	0.79
PI 398,479	0.15
PI 417,440	0.41
PI 417,193	0.29
PI 417,288	0.08
PI 417,359	0.83
Pershing	0.39

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2) Screening specialty soybean cultivars for harvest index.

Soyfood industries have shown interest in specialty varieties. In order to meet the demand for high yield of specialty soybean varieties breeding programs, suitable parents are required. Harvest Index (HI), the proportion of biological yield represented by economic yield, has been found to be a useful predictive index of yield in breeding selection programs for high yields in field crops, e.g., wheat, barley, peas, and dry beans (Donald, 1982; VanDobben, 1962; Thorne, 1958; Watson et al., 1958, 1963; Stinson and Moss, 1960; Sowell et al., 1961; Wallace and Munger, 1966). Improvement in HI of soybean is thus expected to improve the yield of specialty beans. Identification of germplasm with high harvest index will be needed for a breeding program to improve the yield of specialty soybeans.

The objective of our work is to evaluate HI in selected soybean germplasm and identify suitable parents for a breeding program.

Materials and methods: Forty six soybean plant introductions (PIs) in maturity (MG) groups III, IV, and V, classified into small and large seed types, were planted on June 6, 1989, in single-row plots 5.0 m long and 0.75 m apart in a randomized complete block replicated four times. Plots were maintained by hoeing and irrigating whenever necessary. At maturity, 1.0 meter segments of plants were cut at 0.1 m above ground level. A subsample was taken from each harvested sample, dried in an oven at 45 C for 14 hours, weighed after drying, threshed and seed from the subsample was weighed. Harvest index was calcuated as

$$\frac{\text{Weight of seed}}{\text{Total weight of unthreshed plants}}$$

Experimental results: HI data for each cultivar for maturity groups III, IV, and V are given in Tables 1-5.

The range of HI in maturity group II cultivars varied from 0.41 to 0.52. Cultivars 'Kim' and 'Oakland' gave the highest HI and 'Jura' had the lowest HI out of the two cultivars (Table 1)

Cultivars in maturity group IV were separated into two groups according to seed size, large seeded or small seeded. Data for small seeded cultivars are given in Table 2, for large seeded cultivars in Table 3. Among the small seeded cultivars, the HI ranged from 0.44 to 0.57. The highest HI was obtained from cultivar 'Kingston' and lowest from PI 82,264. Among the large seeded cultivars (Table 3) HI ranged from 0.41 to 0.69 and cultivar 'Jefferson' had the highest HI. Cultivar 'Aoda' gave the lowest HI.

Cultivars in maturity group V were separated into two groups of small and large seeded types. As Table 4 indicates, the HI in small seeded group of MG V ranged of 0.33 to 0.53. The highest HI was obtained from cultivar 'Pershing' and lowest from PI 417,052. Among the large seeded cultivars (Table 5) HI ranged from 0.37 to 0.49, and PI 416,467 had the highest HI, while PI 417,440 gave the lowest HI. Comparison between MG III of entries in 1988 and 1989 indicates close agreement for each entry in the two years.

Our study similar to the previous year (Joshi et al., 1989) indicates that there is opportunity to select for high HI within the existing soybean germplasm to develop high seed yield varieties of specialty soybean

Table 1. Harvest index for large seeded entries in Maturity Group III of soybean.

Cultivar	1988 HI	1989 HI	$\bar{X}$ of HI
Kim	0.58	0.52	0.55
Oakland	0.56	0.52	0.54
Willomi	0.55	0.52	0.53
Williams 79	0.55	0.50	0.52
Kanrich	0.57	0.50	0.53
Pella	0.58	0.49	0.53
Guelph	0.54	0.48	0.51
Columbia	0.51	0.47	0.49
Fuji	0.55	0.46	0.50
Kura	0.50	0.41	0.45
Wolverine	0.55	0.41	0.48
		NS	

NS=Differences between cultivars are not significant.

Table 2. Harvest index for small seeded entries in Maturity Group IV of soybean.

Cultivar	1988 HI	1989 HI#	$\bar{X}$ of HI
Kingston	0.56	0.57	0.56
PI 85,505	0.44	0.55	0.49
PI 339,984	0.52	0.55	0.53
Wilson-5	0.45	0.50	0.47
Peking	0.46	0.49	0.47
PI 86,103	0.46	0.46	0.46
Sooty	0.47	0.46	0.46
Norredo	0.51	0.45	0.48
PI 82,264	0.55	0.44	0.49

Differences between cultivars are significant at 0.05 level.

Table 3. Harvest index for large seeded entries in Maturity Group IV of soybean.

Cultivar	1988 HI	1989 HI#	$\bar{X}$ of HI
Jefferson	--	0.69	0.69
Emerald	0.48	0.54	0.51
Kailu	0.55	0.53	0.54
Sanga	0.58	0.52	0.55
Ware	0.49	0.52	0.50
Funk Delicious	0.50	0.52	0.51
Emperor	0.49	0.50	0.49
Shiro	0.54	0.50	0.52
Sata	0.54	0.49	0.51
Kahala	0.55	0.44	0.49
Verde	0.45	0.42	0.43
Hahto/Michigan	--	0.42	0.42
Aoda	0.53	0.41	0.47

#=Differences between cultivars are significant at the 0.5 level.

Table 4. Harvest index for small seeded entries in Maturity Group V of soybean.

Cultivar	1988 HI	1989 HI#	$\bar{X}$ of HI
PI 416,467	---	0.49	0.49
PI 417,159	---	0.47	0.47
PI 417,359	---	0.42	0.42
PI 423,758	---	0.41	0.41
PI 417,288	---	0.40	0.40
PI 417,440	---	0.37	0.39

#=Differences between cultivars are significant at the 0.5 level.

Table 5. Harvest index for large seeded entries in Maturity Group V of soybean.

Cultivar	1988 HI	1989 HI#	$\bar{X}$ of HI
Pershing	---	0.53	0.53
PI 408,155	---	0.47	0.47
PI 423,759	---	0.44	0.44
PI 416,771	---	0.43	0.43
PI 398,479	---	0.41	0.41
PI 423,827	---	0.38	0.38
PI 417,052	---	0.33	0.33

#=Differences between cultivars are significant at the 0.05 level.

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1) Multiple insect resistance in a soybean germplasm line.

Introduction: Insect resistance in soybean has become an important objective in many breeding programs in the southern USA. Van Duyn et al. (1971) discovered high levels of resistance to the Mexican bean beetle (MBB) (Epilachna varivestis Mulsant) in germplasm lines PI 171,451 ('Kosamame'), PI 227,687 ('Miyako White'), and PI 229,358 ('Sodendaizu'). These germplasm lines were later found to be resistant to the soybean looper (SBL) (Pseudoplusia includens Walker) by Turnipseed and Sullivan (1976), the cabbage looper (Trichoplusia ni Huber) by Luedders and Dickerson (1977), and the velvetbean caterpillar (VBC) (Anticarsia gemmatalis Hubner) by Kilen and Lambert (1986). The broad range of resistance exhibited by these lines has enhanced their usefulness in breeding programs. However, a concern over the small gene pool for insect resistance, and the addition of new accessions to the germplasm collection, have prompted systematic insect resistance evaluations of accessions not previously screened. Recently, Kraemer et al. (1988) evaluated all previously unscreened germplasm lines in maturity groups (MG) VI, VII, and VIII for resistance to MBB. In that study, 26 lines not previously screened had substantially lower percent defoliation by MBB than the susceptible checks.

To be useful in several breeding programs, it would be desirable to know if the lines with resistance to MBB were also resistant to other insect species. The objective of the current study was to evaluate the 26 germplasm lines for resistance to SBL and VBC, two major defoliators of soybean in the southern USA.

Materials and methods: The 26 soybean germplasm lines having resistance to MBB were evaluated for resistance to VBC and SBL. Evaluation for VBC resistance was conducted in a greenhouse during the winter of 1987-88. The three insect-resistant germplasm lines identified by Van Duyn et al. (1971) and other known resistant and susceptible checks were also included. 'Centennial', 'Braxton', and 'Kirby' were included as susceptible check cultivars with accessions from MG VI, VII, and VIII, respectively. The resistant check for MG VI was 'Lamar' (Hartwig et al., 1989), and for MG VII and VIII was D75-10169 (Hartwig et al., 1984). Fifteen seeds from each entry were planted in a 1:1:1 mixture of soil:sand:organic peat placed in 20-cm pots, with three pots for each entry. Pots were arranged on greenhouse benches in a randomized complete block design. Photoperiod was extended to 15 h with fluorescent lamps to maintain the plants in a vegetative state. Night temperature was maintained at 24 $\pm$ 4 C and day temperatures ranged from 20 to 30 C. Plants were thinned to 10 per pot. When plants were at the V4 developmental stage (Fehr et al., 1971), five neonate (<4-h old) larvae were placed on the youngest trifoliolate leaf of each plant. After 12 days of feeding, when plants of the susceptible check cultivars were about 80% defoliated, all plants were visually rated for feeding damage on a scale of 1 to 5 (1=0 to 20% defoliation, 2=21 to 40%, 3=41 to 60%, 4=61 to 80%, and 5=>80%). All the entries evaluated for VBC resistance were also evaluated for leaf-feeding damage by SBL in a large field cage described by Lambert (1984).

The entries were planted in a randomized complete block design with three replications. Fifteen seeds from each entry were planted in hills 40 cm apart in rows 76 cm apart. Plants were thinned to 10 per hill. When plants were at the V9 developmental stage, 5000 pairs of velvetbean caterpillar moths were released inside the cage. These moths deposited eggs on plants and a larval population subsequently developed. Plants were rated visually for feeding damage when plants of the susceptible check were at least 80% defoliated, using the same 1 to 5 scale used in the greenhouse evaluation.

Results and discussion: Table 1 gives the mean ratings for leaf feeding of VBC and SBL. None of the MG VI accessions had a rating for both insect species as low as the rating for Lamar. None of the MG VII accessions had a combined rating as low as D75-10169, PI 171,451, or PI 229,358. Although PI 332,690 had a rating for VBC that equalled that for PI 229,358, the rating for SBL was much greater than that for PI 229,358. The line that seemed to have the highest level of resistance to both VBC and SBL was PI 417,061, in MG VIII. In these evaluations, the ratings for this accession were very similar to those for D75-10169.

Crosses have been made between PI 417,061 and Lamar, D75-10169, PI 171,451, and PI 227,687, to determine if the same or different genes are controlling insect resistance. Further evaluation of PI 417,061 is needed to verify these preliminary results and to determine if it has the broad resistance to multiple insect species that is found in the previously identified germplasm accessions.

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Table 1. Response of soybean germplasm accessions and check genotypes to leaf feeding by velvetbean caterpillar and soybean looper.

Entry	Maturity group	Leaf-feeding	
		Velvetbean caterpillar	Soybean looper
		-------Rating ± Standard error---------	
Centennial	VI	4.3±0.33	5.0±0.00
Lamar		2.3±0.33	1.3±0.33
FC 31665		4.3±0.33	3.7±0.33
PI 36,906		2.7±0.33	3.3±0.17
PI 379,621		2.7±0.33	4.3±0.67
PI 416,895		4.0±1.00	3.5±0.50
PI 416,925		4.3±0.67	3.7±0.33
PI 416,937		4.3±0.67	2.7±0.44
PI 417,310		4.3±0.33	4.3±0.33
PI 417,422		3.7±0.88	3.2±0.17
PI 417.427		2.0±0.00	4.0±0.00
PI 423,907		3.3±0.88	4.7±0.33
Braxton	VII	4.3±0.33	4.0±0.00
D75-10169		4.3±0.33	1.0±0.00
FC 31927		4.7±9.33	3.0±0.29
PI 171,451		2.0±0.58	2.0±0.29
PI 200,506		5.0±0.00	4.3±0.33
PI 200,523		4.5±0.50	3.8±0.17
PI 229,321		4.0±0.58	3.2±0.60
PI 229,358		2.3±0.33	1.7±0.17
PI 322,690		2.3±0.88	3.0±0.50
PI 393,547		4.3±0.33	4.2±0.44
PI 416,900		4.7±0.33	2.2±0.44
Kirby	VIII	4.7±0.33	4.7±0.33
D75-10169		2.3±0.33	1.3±0.17
Avoyelles		4.0±0.58	3.8±0.17
PI 159,924		2.7±0.33	4.3±0.33
PI 181,697		3.3±0.33	5.0±0.00
PI 227,687		3.7±0.33	3.7±0.17
PI 324,068		4.7±0.33	3.2±0.33
PI 374,161		4.7±0.33	4.8±0.17
PI 416,806		4.7±0.58	4.2±0.60
PI 417,061		2.0±0.00	1.8±0.33
PI 417,124		5.0±0.00	4.0±0.00
PI 417,136		4.7±0.33	4.0±0.58

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1) Re-examination of the recombination frequency between acid phosphatase and the Kunitz trypsin inhibitor loci in soybean.

Biochemical characters, especially enzymes, have been used increasingly to test linkage relationships in soybean. Hildebrand et al. (1980) first reported Ap (acid phosphatase locus) to be linked to Ti (the soybean Kunitz trypsin inhibitor locus), with a recombination frequency of 16.2 ± 5%. Another linkage of biochemical loci between Ap and Lap1 (leucine aminopeptidase locus 1) was reported thereafter (Kiang et al., 1985). A subsequent study showed that Ti was also linked to Lap1 (Kiang and Chiang, 1986). In mapping the three loci, Kiang (1987) found that the frequency of recombination between Ap and Ti was 6.6 ± 5%. Using the wild soybean (Glycine soja Sieb. and Zucc.) and the hybrid of the wild and cultivated soybeans, Chiang and Kiang (1987) obtained a recombination frequency of 9.19 ± 0.47% between the Ap and Ti loci. Although the latter two values are slightly different, they are significantly different from that reported by Hildebrand et al.

Since PI 87,525 used by Hildebrand et al. was not included in the latter two experiments (Chiang and Kiang, 1987; Kiang, 1987), we thought that PI 87,525 might be unique and that the positions of the Ap and Ti loci in the chromosome of PI 87,525 might be different from those in other soybean cultivars. In this study, we re-estimated the recombination value between Ap and Ti loci by using 'Ebony' and PI 87,525, the same cultivars used by Hildebrand et al. (1980).

Materials and methods: Seeds of Ebony and PI 87,525 were obtained from Dr. R. L. Bernard, curator of the Northern Soybean Germplasm Collection at Urbana, IL. They were planted in the university greenhouse, and the reciprocal crosses were made in 1987. F2 seeds were harvested for electrophoresis in 1988 from the selfed F1 plants also grown in the greenhouse.

Sample preparation, electrophoresis and staining followed the methods developed in our laboratory (Kiang and Chiang, 1986; Kiang et al., 1985). The recombination frequency and homogeneity were estimated by the maximum likelihood method (Allard, 1956).

Results and discussion: A total of 489 F2 seeds were examined from the reciprocal crosses between Ebony and PI 87,525. The segregations of codominant alleles within all individual loci did not significantly deviate from the 1:2:1 ratio (Chi-square test).

The Chi-squares for deviations from the 1:2:1:2:4:2:1:2:1 for independent segregation for the dihybrid were significant in both the reciprocal crosses (Table 1). Since the homogeneity test indicated that the reciprocal crosses were homogeneous (X^2=1.2, 0.50>P>0.25, df=1), they were pooled together for calculating the recombination frequency. The recombination frequency between Ap and Ti was 6.1 ± 0.8%. This estimate is similar to those

in our two previous reports (Chiang and Kiang, 1987; Kiang, 1987), and is significantly different from 16.1 ± 1.5% reported by Hildebrand et al. (1980). Therefore, we conclude that PI 87,525 is not different from other cultivars in terms of the recombination frequency between Ap and Ti.

In the Chiang and Kiang's experiment (1987), a slightly different recombination value, 9.19 ± 0.47%, was obtained. However, heterogeneity existed among the four crosses. Recombination values in two crosses were similar to those reported by Kiang (1987) and in the present study. Wild soybeans used in crosses might be responsible for the slight deviation. Therefore, based on our experiment results, six percent appears to be a consistent estimate of recombination frequency between acid phosphatase and the soybean Kunitz trypsin inhibitor loci.

Different environmental conditions and cultivars used have been offered as explanations of different recombination values observed in lima beans (Allard, 1956). However, recent evidence suggests that environment and genotypes used have no consistent effect on recombination frequencies (Griffin et al., 1989; Pfeiffer and Vogt, 1989). Only chance error exists, and it can be more than 10%. Thus, the different recombination values between the Ap and Ti loci obtained in our laboratory and by Hildebrand et al. (1980) may partly be due to this factor. In addition, different electrophoretic methods may affect the results. Further independent studies from other laboratories are needed.

Table 1. Observed and expected segregation data of F2 seeds from reciprocal crosses between Ebony (Ap-a/Ap-a Ti-a/Ti-a) and PI 87,525 (Ap-c/Ap-c Ti-b/Ti-b).

Cross	Genotype	F2 segregates			Total	X^2*	P
		Ap-a/Ap-a	Ap-a/Ap-c	Ap-c/Ap-c			
Ebony x PI 87525	Ti-a/Ti-a	54(15.6)^	4(31.1)	0(15.6)	58	311.9	>0.001
	Ti-a/Ti-b	7(31.1)	111(62.3)	13(31.1)	131		
	Ti-b/Ti-b	0(15.6)	9(31.1)	51(15.6)	60		
	Total	61	124	64	249		
PI 87525 x Ebony	Ti-a/Ti-a	46(15)	5(30)	0(15)	51	367.3	>0.001
	Ti-a/Ti-b	9(30)	103(60)	5(30)	117		
	Ti-b/Ti-b	0(15)	6(30)	66(15)	72		
	Total	55	114	71	240		

* Chi-square of deviation from the 1:2:1:2:4:2:1:2:1 independent segregation ratio for the dihybrid segregates minus the Chi-squares of deviation from the 1:2:1 segregation ratio at the two individual loci involved (df=8-2 - 2=4).

^ Expected numbers are in parentheses.

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1) Harvest index and related characteristics of small and large seeded soybean genotypes.

A quantitative measure of biological efficiency of a crop plant is the harvest index (defined as ratio of the economic product to the total phytomass). Donald and Hamblin (1976) concluded that harvest index (HI) may be used as a criterion for yield evaluations. The association of increased yield with increased HI in wheat has been demonstrated by Austin et al. (1980) and Feyerherm et al. (1984). Gent and Kiyomoto (1989) reported that greater HI of semi-dwarf wheat cultivars was related to greater partitioning of current photosynthate to the grain.

In soybean, as in all crop plants, study of HI is important for the formulation of breeding and agronomic strategies for increasing the biological efficiency of the soybean plant. The increasing demand for specialty soybeans mandates the evaluation of large and small seeded soybean germplasm. Bhardwaj and Bhagsari (1989) presented preliminary results from unreplicated experiments (augmented design) conducted with large and small seeded soybean genotypes and observed that HI was positively correlated with yield among small seeded genotypes but not among large seeded genotypes.

Our objectives were to study variation for harvest index, yield, leaf area index (LAI), phytomass, and height in small and large seeded soybean genotypes and to ascertain the effects of differing seed sizes on these characteristics.

Materials and methods: Three separate experiments were conducted during 1989 at the Agricultural Research Station, Fort Valley State College. Experiment 1 consisted of 15 soybean genotypes, five each from the large seeded group (100-seed-weight 18 g or more), medium seeded group (100-seed-weight between 10 and 18 g), and small seeded group (100-seed-weight 10 g or less). Experiments 2 and 3 consisted of 35 small seeded and 21 large seeded genotypes, respectively. Experiment 1 was planted May 25, 1989, in an RCBD design with 4 replications with 6-m long and 0.9-m wide 4-row plots. Experiments 2 and 3 were planted May 26, 1989, in RCBD design with 3 replications with 4.5-m long and 0.9-m wide 3-row plots. In Experiment 3, all plots of PI 417159 had inadequate stand. Consequently, statistical analysis was based only on 20 genotypes.

Plant samples were taken approximately 80 days after planting by harvesting 0.3 m of row length from the middle row of each plot at ground level. The plants were separated into leaves, stems, and pods. The leaf area was recorded by using Li-Cor 3000, automatic leaf area meter. At final harvest (from Oct. 5 to Nov. 14, 1989), two samples, each 1 m long, were harvested from the middle row(s) of each plot. The plant material was dried to a constant weight. The total weight of dry above-ground material was recorded

before thrashing of samples with a plot combine. After thrashing, the seed were cleaned and weighed. The apparent harvest index was determined as: seed weight/total dry weight of above-ground material*100. Phytomass was calculated from total dry weight of above-ground material. The leaves and petioles had fallen off the plants at final harvest. All data were subjected to analysis of variation with mean separation based on least significant difference with 5% level of significance.

Results and discussion: A comparison of different groups on the basis of seed size (Table 1) indicated that small- and medium-seeded groups had a significantly higher harvest index and a significantly lower LAI as compared with the large-seeded group. The yield, phytomass, and height differences among these groups were not significant. Yield exhibited significant positive correlation with LAI and height in all three groups. Harvest index was not related to yield but exhibited significant negative correlation with phytomass and height in the medium-sized group. The lack of relationship between yield and harvest index was surprising during 1989. A significant positive correlation (r=0.62) between yield and harvest index was observed during 1988 from an experiment with these genotypes. Apparently environment is an important factor and needs to be given serious consideration in such experiments.

Significant variation existed among 35 small-seeded genotypes in Experiment 2 for harvest index, yield, leaf area index, phytomass, and plant height (Table 2). The harvest index ranged from 27.63 (for PI 227687) to 52.13 (for N83-640) whereas yield ranged from 1.56 (for PI 417193) to 4.00 MG/ha (for PI 423827B). A significant positive correlation (Table 4) was observed between yield and harvest index. The yield was positively correlated to leaf area index, phytomass, and height, whereas harvest index exhibited a significant negative correlation with leaf area index, phytomass, and height. The presence of significant variation for yield and harvest index and a positive correlation between these two traits indicates that biological efficiency and higher productivity are not mutually exclusive and improvement in these characteristics is possible. N83-640 was observed to be biologically most efficient, whereas PI 227687 was observed to be the biologically least efficient genotype.

Existence of significant variation for all characteristics under study in Experiment 3 (large-seeded genotypes) was observed (Table 3). Harvest index ranged from 24.51 to 48.78, and yields ranged from 0.80 to 3.02 MG/ha. PI 417288 was observed to be the biologically most efficient genotype. PI 417213 had the lowest yield (0.80 MG/ha) and was least efficient (harvest index=24.51). A significant positive correlation (Table 4) between yield and harvest index (r=0.36). Yield had a significant positive correlation with leaf area index, phytomass, and plant height. However, harvest index was not related to leaf area index or phytomass but exhibited a negative correlation with plant height.

Conclusions: Significant variation was observed in available germplasm for harvest index, yield, leaf area index, phytomass, and plant height to facilitate selection for desired combinations of these traits in both large- and small-seeded groups of genotypes. Small- and medium-seeded genotypes had a higher harvest index and lower leaf area index as compared to large-seeded genotypes. The yield differences among small-, medium-, and large-seeded

groups were not significant. Yield and harvest index were positively related in both small-seeded and large-seeded genotypes, indicating possibility of simultaneous improvement in these traits. It seems that environmental variation might be important when considering long range comparisons and should be given consideration.

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Table 1. Harvest index (HI), yield, leaf area index, phytomass at final harvest, and height of soybean genotypes as affected by seed size during 1989.

Seed size	HI (%)	Yield MG/ha	LAI	Phytomass MG/ha	Height cm
Small	42.97 A	2.67	2.99 B	6.26	52.95
Medium	42.87 A	2.98	3.44 B	7.08	57.30
Large	36.95 B	2.72	4.39 A	7.35	60.00

Mean separation of seed sizes was based on LSD (0.05).

Table 2. Harvest index (HI), yield, leaf area index, phytomass at final harvest, and height of small-seeded soybean genotypes during 1989.

No.	Genotype	HI (%)	Yield MG/ha	LAI	Phytomass MG/ha	Height cm
1	N83-640	52.13	2.96	3.85	5.67	43.33
2	H86-5709	51.01	2.32	1.94	4.56	35.33
3	D59-2537	50.80	3.33	5.28	6.61	46.00
4	PI 417052	50.29	1.86	4.29	3.70	60.67
5	PI 423852	48.54	2.79	3.42	5.76	48.00
6	PI 398269	47.75	1.98	2.82	4.15	27.67
7	PI 423759	47.26	2.28	3.52	4.95	42.67
8	Rocky	46.73	2.09	3.59	4.52	42.67
9	H86-5584	46.29	2.76	5.76	5.93	45.00
10	Pershing	46.08	2.10	2.86	4.59	34.67
11	PI 423827B	45.79	4.00	4.88	8.70	50.00
12	PI 416867	45.62	3.24	5.02	7.11	46.00
13	H86-5984	45.51	2.33	3.94	5.12	46.33
14	PI 408155	44.69	2.33	2.40	5.32	29.00
15	Vance	44.68	1.82	1.98	4.05	30.33
16	PI 196177	44.42	1.73	0.72	3.78	24.00
17	PI 417193	44.35	1.56	3.81	3.51	23.00
18	PI 494181	44.32	2.16	2.57	4.90	43.67
19	H86-5824	44.28	2.87	4.79	6.48	57.33
20	H86-5708	43.92	2.77	3.71	6.31	45.67
21	PI 398479	43.77	2.21	3.49	5.06	44.00
22	PI 86490	41.78	2.49	6.16	5.95	63.33
23	PI 171437	40.79	1.86	3.30	4.59	54.67
24	PI 398583	38.33	2.78	2.73	7.23	27.33
25	PI 194773	37.79	2.11	3.94	6.34	85.33
26	PI 416900	35.84	2.91	4.46	8.22	124.00
27	Laredo	35.38	2.41	6.58	6.81	97.33
28	PI 427241	34.96	1.64	3.72	4.71	69.33
29	PI 416771	33.10	0.89	2.22	2.67	21.00
30	PI 324068	32.04	2.50	5.32	7.81	85.33
31	PI 393547	31.27	2.61	6.81	8.55	111.33
32	PI 222397	30.75	1.87	3.15	6.09	61.33
33	PI 181697	29.17	2.68	6.21	8.99	94.00
34	PI 393550	28.37	2.27	6.38	8.10	103.33
35	PI 227687	27.63	2.43	7.34	8.85	136.67
	Mean	41.58	2.37	4.08	5.87	57.03
	LSD (0.50)	9.37	0.78	2.11	1.95	11.17
	CV	13.83	20.28	31.76	20.40	12.02

Table 3. Harvest index (HI), yield, leaf area index, phytomass at final harvest, and height of large-seeded soybean genotypes during 1989.

No.	Genotype	HI (%)	Yield MG/ha	LAI	Phytomass MG/ha	Height cm
1	PI 417288	48.78	1.94	2.27	3.99	34.33
2	PI 423758	46.03	1.99	3.21	4.37	40.33
3	PI 417128	44.96	2.74	3.40	6.14	57.33
4	Tokyo	43.84	3.02	4.84	6.89	57.00
5	PI 181565	43.53	2.71	3.76	6.25	55.00
6	Hahto	41.89	1.65	2.72	3.97	38.67
7	PI 416982	40.82	1.72	2.52	4.23	34.33
8	PI 417359	40.66	1.51	2.83	3.66	41.67
9	PI 417440	40.56	1.62	1.32	4.00	35.67
10	PI 416893	39.44	2.47	2.89	6.24	60.67
11	Rockusun	39.42	2.71	3.64	6.90	57.67
12	D71-V89	37.11	2.78	5.32	7.55	60.00
13	PI 417310	36.89	1.85	3.57	5.10	40.33
14	D71-V86	36.68	2.31	5.12	6.23	60.33
15	PI 230972	36.26	2.22	3.97	6.12	55.33
16	D74-9820	35.73	2.69	6.70	7.39	69.00
17	H86-8097	35.37	1.91	3.38	5.49	73.67
18	Tanbagura	35.16	2.75	4.83	7.82	57.33
19	H86-6865	35.01	2.29	4.03	6.45	66.00
20	PI 417213	24.51	0.80	2.51	3.26	45.00
	Mean	39.13	2.18	3.64	5.60	51.98
	LSD (0.50)	5.82	0.76	1.84	1.74	10.87
	CV	8.99	21.05	30.61	18.81	12.65

Table 4. Correlations between various characteristics of small-seeded and large-seeded soybeans during 1989.

	Small seeded		Large seeded	
Characteristic	HI	Yield	HI	Yield
Harvest index (%)	--	0.26*	--	0.36**
Yield (MG/ha)	0.26*	--	0.36**	--
LAI	-0.24*	0.36**	-0.24	0.43**
Phytomass (MG/ha)	-0.44**	0.72**	-0.07	0.89**
Height (cm)	-0.59**	0.21**	-0.29*	0.44**

*, ** Correlations significant at 5% and 1% level, respectively.

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1) Evaluation of vegetable soybean genotypes for nutritional and antinutritional factors.

Introduction: Consumers across the US concerned with health and physical fitness are exploring for an alternative vegetable crop that can be incorporated into a low calorie nutritional diet. Vegetable-type soybean is already popular as a food in the Orient, where the incidence of heart disease is low. Also, vegetable-type soybean is different from the mature soybean in flavor and texture (Morse, 1950) and are low in trypsin inhibitor (Gupta and Deodhar, 1975). Therefore, one of the major thrusts of the current plant breeding research at Virginia State University (VSU) is the development of vegetable soybean cultivars with desirable agonomic traits and high nutritional quality and quantity.

Table 1. Mean percentage of protein (16% N) and oil, and trypsin inhibitor and lipoxygenase activities in selected vegetable soybean genotypes.

Trypsin genotype	Protein	Oil	Lipoxygenase	Inhibitor
	------%--------		units/mg meal	units/mg
Ware	43.49	19.01	3787.23	32.42
Emperor	46.50	17.87	2291.59	32.68
Sango	38.35	23.36	2525.39	34.85
Kingston	46.78	18.42	2423.66	31.34
Sooty	47.87	19.25	1143.12	29.37
Wilson-5	47.83	15.65	3011.84	28.30
PI 416982	44.81	19.83	3164.54	19.83
PI 416771	46.76	17.64	1270.83	24.71
PI 417288	42.18	18.54	1505.48	27.69
PI 417322	45.83	17.24	1238.60	27.50
PI 417052	41.18	20.95	1559.13	30.79
PI 417213	42.73	19.06	1831.10	33.08
PI 417310	43.88	18.94	0819.81	28.70
PI 423759	38.94	22.40	4750.42	33.55
PI 423582	40.22	15.15	1688.57	25.08
PI 222397	36.92	22.26	1602.57	47.06
PI 171437	41.79	20.25	2373.60	29.67
C.V. %	01.18	02.64	01.80	01.63
LSD (0.05)	00.85	00.84	65.22	00.85

Materials and methods: Seventeen vegetable soybean genotypes, six from maturity groups (MG) IV and V, and five from MG VI were selected on the basis of seed size, MG, and seed availability during the time of the experiment.

Three replications of each entry were planted in four-row plots, arranged in randomized complete block design, at Randolph Research Farm of VSU, Petersburg, VA. Each four-row plot was 4 m long and 3.60 m wide, with the spacing of 0.90 m between rows; a seeding rate of 23 seeds per meter was used. At maturity (R8) (Fehr et al., 1971) each entry was evaluated by harvesting the two center rows of each plot. Seed samples were taken and ground. The samples were analyzed for percent of protein, percent oil, trypsin inhibitor (TI) and lipoxygenase activities, according to the procedures described by AOAC (1980), Kinsalla et al. (1977), Kakade et al. (1969), and Hafez et al. (1985), respectively. Data were statistically analyzed and means were separated, by using Least Significant Difference (LSD) test at the 5% level of significance.

Results and discussion: The vegetable-type soybean genotypes studied in this experiment showed significant differences for percent protein, percent oil, trypsin inhibitor and lipoxygenase activities. The mean protein content of the genotypes tested was 43.32%, and ranged from 36.92 to 47.87% (Table 1). Two cultivars, 'Sooty' and 'Wilson-5', both from MG IV, had the highest percent protein and PI's 222397 and 423759, from MG VI, had the lowest. Wide variations in percent oil and content was found. The mean oil content was 18.44% and ranged from 17.24 to 23.36%. In our study a moderately significant negative correlation (r=-0.588**) was observed between the protein and oil content. This result was in agreement with the data reported by Hafez (1983). Trypsin inhibitor and lipoxygenase activities are two of the anti-nutritional factors that are prevalent in soybean. Especially, lipoxygenase is responsible for the off-flavor in soybean food and beverages (Sessa, 1977). This study reveals that the overall mean of lipoxygenase activity in the vegetable cultivars (2530.46 units/min/mg meal) was significantly higher than the overall mean of the PI's, which is 1982.15 units/mn/mg meal. Among the genotypes tested, PI 417310 (MG VI) had the lowest lipoxygenase activity, while the cultivar 'Ware' (MG IV) had the highest. The trypsin inhibitor activity among the genotypes tested ranged from 24.71 to 34.85 units/mg meal, which was similar to that reported by Gupta and Doedhar (1975). The PI 229397 (MG VI) had the highest trypsin inhibitor activity, while PI 416777 (MG VI) had the lowest. From the foregoing discussion of the results obtained in the present study, it could be inferred that great genetic variation exists among the genotypes tested, for percent of protein, oil, trypsin inhibitor, and lipoxygenase activities, which could be incorporated with other cultivars with desirable agronomic traits, through hybridization and selection.

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2) Evaluation of vegetable soybean genotypes for phytate phosphorus and minerals.

Introduction: Phytic acid (PA), myo-inositol 1,2,3,4,5,6-hexaphosphate, is the storage form of phosphorus (Pi) in seeds (Cheryan, 1980). The PA binds with nutritionally important metals, especially Zn and Ca, possibly contributing to nutritional deficiencies in nonruminant animals. The growing interest in vegetable soybean as a source of vegetable protein has spurred an interest in its PA content. Several studies have shown significant varietal differences in the accumulation of phytate. If significant variation in seed PA and minerals exists among the vegetable soybean genotypes, a breeding program aimed at reducing the PA and increasing the mineral content of vegetable soybean seeds should be feasible. Therefore, one of the major thrusts of current plant breeding research at Virginia State University is to develop vegetable soybean cultivars with high nutritional quality and quantity.

Materials and methods: Seventeen vegetable soybean genotypes, six from maturity groups (MG) IV and V, and five from MG VI were selected on the basis of seed size, MG, and seed availability during the time of the experiment. Three replications of each entry were planted in four-row plots, arranged in randomized complete block design, at Randolph Research Farm of Virginia State University, Petersburg, VA. Each four-row plot was 4 m long and 3.6 m wide, with space of 0.9 m between rows. A seeding rate of 23 seeds per meter was used. Plants were harvested at maturity (R8) (Fehr et al., 1971) and each entry was evaluated by harvesting the two center rows of each plot. Seed sample was collected and ground. The samples were analyzed for phytate (PA), total phosphorus (TPi), available phosphorus (APi) according to Mohamed et al. (1986) and Hafez et al. (1989). Ca, K, Fe, Cu, and Mn were determined as described in AOAC (1984). Data were statistically analyzed and means were separated by using least significant difference (LSD) test at the 5% level of significance.

Results and discussion: The 17 vegetable genotypes used in this study showed significant differences for phytate, total phosphorus, and available phosphorus. The mean of phytate content of tested genotypes was 31.31 mg/gm meal (Table 1). The genotypes 'Kingston' (MG IV) and PI 417,052 (MG V) had the highest phytate content, while 'Emperor' (MG IV) and PI 416,771 (MG V) had the lowest. Wide variation also was found in total phosphorus (TPi). The mean of TPi was 20.82 mg/gm meal. The cultivar 'Sango' and PI 417,213 had the highest Pi and Emperor and PI 417,052 had the lowest. A moderately significant positive correlation (r=0.383) was observed between TPi and PA. This correlation was lower than that reported by Raboy et al. (1984). The data also showed wide variation in available phosphorus (AP) between tested genotypes. The mean of AP was 11.97 mg/gm meal and ranged from 3.78 for PI 417,052 to 18.36 mg/gm meal for Sango. Strong positive correlation coefficient (r=0.900) was found between TPi and AP. However, no correlation was found between PA and AP. Calcium (Ca), potassium (K), iron (Fe), copper (Cu), and manganese (Mn) were analyzed and the means were 2330.61, 116.78, 45.78, 30.98, and 16.17 ug/gm meal, respectively.

Moderately significant negative correlations (r=-0.459, -0.524 and -0.389) were found between phytate and Ca, K, and Mn, respectively. Highly significant negative correlation (r=-0.75) was found between TPi and Cu. Also, Fe and Cu were significantly correlated with AP (R=-0.54 and -0.664, respectively). Between analyzed minerals, a moderate positive correlation (r=586) was found between Ca and K. Similarly, significant positive correlations (r=0.339 and 0.567) were found between K and Cu, and between Fe and Cu. From the foregoing discussions, it could be inferred that great variation exists among the tested genotypes for PA, TPi, AP, Ca, K, Fe, Cu, and Mn, which could be incorporated with other cultivars with desirable agronomic traits, through hybridization and selection.

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Table 1. Phytate (PA), total phosphorus (TPi), available phosphorus (APi), Ca, Fe, Mn, K, and Cu of selected vegetable soybean genotypes.

Genotype	MG	Seed size	PA	TPi	APi	Ca	K	Fe	Cu	Mn
			-----mg/g meal----			--------ug/gm meal----------				
Ware	IV	Lg	29.27	18.78	10.55	2817.8	178.4	48.1	34.1	28.2
Emperor	IV	Lg	21.91	12.77	6.61	2113.0	124.1	46.2	49.2	22.2
Sango	IV	Lg	35.45	28.33	18.36	1676.1	85.9	26.5	8.8	5.7
Kingston	IV	Sm	40.05	20.34	8.98	2413.7	101.6	40.5	32.1	5.9
Sooty	IV	Sm	30.95	23.63	14.92	2782.7	128.5	55.7	35.1	10.4
Wilson-5	IV	Sm	38.36	21.60	10.81	1326.9	98.9	50.8	36.1	7.8
PI 416982	V	Lg	25.01	19.20	12.16	2546.8	134.0	42.3	26.4	10.5
PI 417288	V	Lg	31.80	19.69	10.74	1475.8	104.9	51.2	35.5	3.9
PI 417322	V	Lg	32.16	24.38	15.00	1477.2	108.2	35.5	12.2	68.4
PI 416771	V	Sm	18.80	16.06	10.77	3262.9	131.8	79.0	39.4	28.8
PI 417052	V	Sm	40.67	15.22	3.78	1954.3	100.8	67.5	33.5	6.3
PI 423759	V	Sm	26.03	21.04	13.72	2743.9	129.9	44.9	33.7	43.5
PI 417213	VI	Lg	32.89	27.14	17.79	2502.4	118.1	34.3	21.8	9.0
PI 417310	VI	Lg	33.57	20.66	11.12	2468.5	113.8	42.8	36.6	5.5
PI 423582	VI	Lg	30.49	20.24	11.57	2918.6	112.8	40.1	26.0	8.6
PI 222397	VI	Sm	35.60	22.13	12.38	2829.4	111.5	32.1	34.8	4.3
PI 171437	VI	Sm	29.26	22.68	14.58	2310.3	113.0	43.8	31.3	7.3
CV %			1.30	1.68	2.38	0.15	1.26	2.79	0.85	.75
LSDA (0.05)			0.24	0.58	0.11	5.89	0.86	2.12	0.15	0.2

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3) Evaluation of vegetable soybean genotypes for fatty acid composition.

Introduction: Soybean breeding programs traditionally have focused on improvement of agronomic traits. In recent years, however, due to elevated cholesterol levels in the blood stream, a condition associated with high risk of heart disease, research emphasis has been placed on nutritional quality improvement. Research findings suggested that the composition and quantity of fats in the diet are critical to control cholesterol levels in the blood stream. Thus another aspect of soybean oil improvement would involve changes in fatty acid composition. Fatty acid composition is an important determinant of soybean oil quality. However, it has been found that soybean oil contains more than 6% linolenic acid. This particular fatty acid has been identified as the unstable component of soybean oil that is responsible for the undesirable odors and flavors commonly associated with poor oil quality (Dutton et al., 1951; Smouse, 1979). The objective of this study was to determine the fatty acid profile of selected vegetable soybean genotypes.

Materials and methods: Seventeen vegetable soybean genotypes, six each from maturity groups (MG) IV and V, and five from MG VI were selected on the basis of seed size, MG, and seed availability during the time of the experiment. Three replications of each entry were planted in four-row plots, arranged in randomized complete block design, at Randolph Research Farm of VSU, Petersburg, VA. Each four-row plot was 4 m long and 3.6 m wide, with a spacing of 0.9 m between rows; a seeding rate of 23 seeds per meter was used. At maturity (R8) (Fehr et al., 1971) each entry was evaluated by harvesting the two center rows of each plot. Seed samples were taken and ground. The samples were analyzed for palmitic, stearic, oleic, linoleic, and linolenic fatty acids, according to the procedures described by Kinsella et al. (1977). Data were statistically analyzed and means were separated by using Least Significant Difference (LSD) test at the 5% level of significance.

Results and discussion: Fatty acids, as components of triglycerides, form the largest fraction of soybean oil. Several workers have reported wide variation in fatty acid composition among soybean genotypes and selections. In this study, highly significant differences in the content of fatty acids were observed among the tested genotypes (Table 1). Furthermore, palmitic, oleic, and linoleic acids constituted more than 95% of the total fatty acids measured. Stearic and linolenic acids were present in small amounts. The range of variation among cultivars was smaller than that for PI's, especially for palmitic, stearic, oleic, and linoleic acids.

The fatty acid composition of the vegetable soybeans studied here have a high concentration of linoleic acid (53.34%) and a low, but significant, amount of linolenic acid (9.19%). Both of these acids have a significant effect upon oil quality. High concentration of linolenic acid is undesirable in terms of oil, because it is readily oxidized, which is believed to be the cause of off-flavor in soybean oil (Ho et al., 1978). Even though the high linolenic acid is undesirable in terms of oil stability, it is an essential fatty acid in the mammalian diet. Mammals lack the enzymes needed to produce polyunsaturated fatty acids, hence, these fatty acids must be supplied through external sources. Among the genotypes studied, 'Ware', 'Emperor', and PI 416982 had low linolenic acid content (Table 1). A recent study has provided conclusive evidence that linoleic and linolenic acids are produced by

successive desaturation of oleic acid and that serves to explain the direction and magnitude of correlations among these three fatty acids. In our study the correlations of oleic with linoleic and linolenic were highly significant and

Table 1. Mean percentage of fatty acid composition of selected vegetable soybean genotypes.

Genotype	Palmitic C16:0	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18-3
	----------	----------	%	----------	----------
Ware	12.49	2.97	28.03	43.72	6.07
Emperor	10.71	2.53	25.53	52.95	7.49
Sango	10.09	3.16	16.07	55.05	10.17
Kingston	10.43	3.15	17.66	53.94	12.60
Sooty	8.37	2.83	24.20	53.13	9.31
Wilson-5	10.43	3.80	17.83	55.96	9.21
PI 416982	11.15	3.32	22.67	53.86	7.70
PI 416771	10.69	3.30	25.21	51.57	8.47
PI 417288	10.63	3.81	20.36	50.62	9.48
PI 417322	11.08	3.43	19.28	56.18	9.51
PI 417052	10.35	3.21	29.80	47.21	8.52
PI 417213	12.58	3.37	18.95	54.33	9.77
PI 417310	11.85	3.35	17.40	54.97	10.36
PI 423759	11.52	3.14	16.31	56.83	10.90
PI 423582	11.29	3.38	17.30	54.57	9.24
PI 222397	10.26	2.75	18.08	56.03	8.35
PI 171437	12.31	3.08	16.24	56.14	9.08
C.V. %	1.70	5.89	0.85	13.74	1.44
LSD (0.05)	0.31	0.32	0.30	1.17	0.22

negative, -0.820 and -0.663, respectively. As shown in Table 1, the cultivar Ware and PI 417052 have high concentrations of oleic acid but relatively low concentrations of linoleic and linolenic acids. From this study Ware, a large-seeded vegetable type from MG IV, and PI 417052, a small-seeded type from MG V, have great potential for cultivar development with improved nutritional quality for large- and/or small-seeded types of vegetable soybean.

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1. Genetic relationship among soybean lines resistant to Heterodera glycines races 3 and 5.

Soybean cyst nematode (SCN), a destructive pest on soybean was first reported in the USA in 1954 (Winstead et al, 1955). Sources of resistance to SCN have been identified (Epps and Hartwig, 1972; Anand and Gallo, 1984; Anand et al., 1988). Genetic studies were reported on a few of these resistant sources (Caldwell et al., 1960; Matson and Williams, 1965; Thomas et al., 1975; Hancock et al., 1987; Rao-Arelli and Anand, 1988a, 1988b; Rao-Arelli et al., 1988; Anand and Rao-Arelli, 1989; Rao-Arelli et al., 1989). The purpose of this brief report is to provide additional information on the genetic relationships among soybean plant introductions (PIs) for resistance to SCN races 3 and 5.

Materials and methods: The following crosses were made among resistant soybean PI lines: 1) 90763 x 89772, 2) 88788 x 89772, 3) 437654 x 438489B, 4) 437654 x 89772, and 5) 437654 x 404166.

Crosses 1, 2, and 3 were studied for reaction to SCN race 3, whereas crosses 3, 4, and 5 were evaluated for race 5 reaction. The procedures for evaluation were already described (Rao-Arelli and Anand, 1988a).

Results and discussion: The crosses PI 90763 x PI 89772 and PI 437654 x PI 438489B did not segregate in the F2 for SCN race 3 reaction, indicating that the parents have identical alleles (Table 1). The cross PI 88788 x PI 89772 segregated into 240 resistant (R) against 50 susceptible (S) in the F2 generation (Table 1). This was in good agreement with a 13(R):3(S) ratio (P=0.7-0.8). It suggests that each parent has one dominant and one recessive gene for resistance at different loci.

The cross Peking(R) x PI 88788(R) segregated into 13(R):3(S) ratio in the F2 and 15(R):1(S) in the F3 generations (Rao-Arelli and Anand, 1988). Based on F1, F2, and F3 data, it was indicated that SCN race 3 reaction in PI 88788(R) was conditioned by two dominant and one recessive genes (Rao-Arelli et al., 1988). These data suggest that at least one dominant and one recessive gene in both the parents in are common. The dominant locus conferring resistance in Peking has been designated earlier to the reaction of Missouri SCN population (Matson and Williams, 1965) and probably is in common with one of the two dominant loci in PI 88788. The other dominant locus in PI 88788 conditioning resistance to SCN race 3 needs new designation.

The crosses PI 437654(R) x PI 438489(R), PI 437654B(R) x PI 89772(R), and PI 437654(R) x PI 404166(R) segregated in the F2 generation for reaction to SCN race 5 indicating the genes conferring resistance were located at different loci in these parents (Table 1). All the F1s for these crosses were resistant to SCN race 5.

The F2 population of the cross PI 437654 x PI 438489B segregated into 193(R) compared with 34(S) plants for SCN Race 5 reaction. The segregation fit a 13(R):3(S) ratio (P=0.20-0.05).

The cross between PI 437654(R) x PI 89772(R) segregated into 365 resistant against 15 susceptible in the F2 for SCN race 5 reaction. This was in good agreement with 61(R):3(S) ratio (P=0.50-0.30), indicating each parent had one dominant and two recessive genes conditioning resistance and these were located at different loci.

The cross between PI 437654(R) x PI 404166(R) segregated into 257 resistant compared with 43 susceptible in the F2 generation for SCN race 5 reaction. This was in good agreement with 55(R):9(S) ratio (P=0.95-0.8), indicating each parent has two dominant and one recessive gene at different loci.

These results for both the races will be further tested by using F3 families.

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Table 1. Reaction of F1 and F2 plants from crosses between six different soybean genotypes to SCN Races 3 and 5.

Cross	F1 R/S	F2 R	F2 S	Expected ratio	Chi square	P value
Race 3						
PI 90763 x PI 89772	10(R)	340	0	-	-	-
PI 437654 x PI 438489B	10(R)	360	0	-	-	-
PI 88788 x PI 89772	10(R)	240	50	13(R):3(S)	0.13	0.8-0.7
Race 5						
PI 437654 x PI 438489B	10(R)	193	34	13(R):3(S)	1.96	0.2-0.05
PI 437654 x PI 89772	10(R)	365	15	61(R):3(S)	0.46	0.5-0.3
PI 437654 x PI 404166	10(R)	257	43	55(R):9(S)	0.02	0.95-0.9

R=Resistant; S=Susceptible

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1) Biosystematics of the genus Glycine, 1989.

Table 1 contains the latest version of the species in the genus Glycine, three-letter identification code, 2n chromosome number and genome symbols.

The major points are are follows:

1. Three new wild perennial Glycine species were described by Tindale and Craven (1988). They are G. albicans, G. hirticaulis, and G. lactovirens. The three species were collected from remote areas of northwestern Australia.

A weedy, intermediate annual form between G. max and G. soja occurs in the wild. Skvortzow (1927) named this variant G. gracilis, Hermann (1962) removed 'G. gracilis' from the species rank and incorporated it into G. max. Recent studies by Singh and Hymowitz (1988, 1989) revealed that the three taxa belong to a common gene pool (GG) and should be considered as forms of one species.

Table 1. Species in the genus Glycine Willd., three letter code, somatic chromosome number and genomic symbols, 1989.

Species	Code	2n	Genome symbol
Subgenus Glycine			
G. albicans Tind. and Craven	ALB	40	----
G. arenarea Tind.	ARE	40	----
G. argyrea Tind	ARG	40	A2A2
G. canescens F.J.Herm.	CAN	40	AA
G. clandestina Wendl.	CLA	40	A1A1
G. curvata Tind.	CUR	40	----
G. cyrtoloba Tind.	CYR	40	CC
G. falcata Benth.	FAL	40	FF
G. hirticaulis Tind. and Craven	HIR	80	----
G. lactovirens Tind. and Craven	LAC	40	----
G. latifolia (Benth.) Newell and Hymowitz	LAT	40	B1B1
G. latrobeana (Meissn) Benth.	LTR	40	----
G. microphylla (Benth.) Tind.	MIC	40	BB
G. tabacina (Labill.) Benth.	TAB	40	B2B2
No adventitious roots		80	AAB2B2
Adventitious roots		80	BBB2B2
G. tomentella Hayata	TOM	38	EE
		40	DD
		78	DDEE
		80	AADD
Subgenus Soja (Moench) F.J.Herm.			
G. soja Sieb. and Zucc.	SOJ	40	GG
G. max (L.) Merr.	MAX	40	GG

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2) Management of the USDA wild perennial Glycine collection, 1989.

Under a specific cooperative agreement between the University of Illinois and the USDA, the wild perennial Glycine collection currently is being maintained at the University of Illinois. Table 1 contains the names of the species, somatic chromosome number, number of accessions and distribution.

The major points are as follows:

1. A few seed of three newly described species were received from CSIRO/Canberra, that is, G. albicans, G. hirticaulis, and G. lactovirens. Similar to G. latrobeana, the new species are recalcitrant regarding seed multiplication.

2. Several accessions of G. tomentella were collected on the island of Timor, Indonesia by R. J. Lawn, CSIRO/Brisbane. These are our first accessions from Indonesia. All accessions studied carried 2n=80 chromosomes.

3. During the year, 22 seed requests were received. A total of 497 packets of seed were shipped. Domestically, seed were sent to Alabama, Arizona, Arkansas, California, Delaware, Illinois, and Texas. Internationally, seed were mailed to Australia, France, Japan, People's Republic of China, and South Korea.

4. This past year, the perennial Glycine plants grown in the Turner Hall greenhouse complex or just outside the greenhouse during the summer months were devastated by pests. The following control measures were used with varying degrees of success:

a. Mealy bugs: Insecticidal soap, Orthene, Diazinon, 100% ethanol and cotton swabs, spray 50% ethanol on plants.

b. Spider mites: Avid.

c. White flies: Isotox, Xclude, Yellow sticky strips.
d. Mildews: Benomyl.

Other chemicals used included Resmethrin, Malathion, Knox-off, and Sevin (outside greenhouse).

In late August and early September, the greenhouses were thoroughly cleaned. The windows, benches, light and metal fixtures and the floors were washed. The greenhouses were sealed and fumigated with Sumithren. All pots and bamboo stakes were steamed. The sand bench used for germinating seeds was thoroughly drenched with Banrot.

Table 1. Wild perennial Glycine species, somatic chromosome number, number of accessions and distribution, 1989.

Species	2n	No. of accessions	Distribution
G. albicans	40	1	Australia
G. arenaria	40	3	Australia
G. argyrea	40	3	Australia
G. canescens	40	51	Australia
G. clandestina	40	130	Australia
G. curvata	40	5	Australia
G. cyrtoloba	40	29	Australia
G. falcata	40	16	Australia
G. hirticaulis	80	1	Australia
G. lactovirens	40	1	Australia
G. latifolia	40	41	Australia
G. latrobeana	40	13	Australia
G. microphylla	40	24	Australia
G. tabacina	40	17	Australia
	80	70	Australia, West-Central and S. Pacific Islands, Taiwan
	Unknown	159	
G. tomentella	38	3	Australia
	40	11	Australia, Papua New Guinea
	78	50	Australia, Papua New Guinea
	80	47	Australia, Papua New Guinea Taiwan, Philippines, Indonesia
	Unknown	147	

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1) Resistance to Phytophthora megasperma f. sp. glycinea in the soybean PI 437,833.

Phytophthora root rot, caused by Phytophthora megasperma f. sp. glycinea Kuan and Erwin, (Pmg) is one of the most serious diseases of soybean when conditions are favorable (Kuan and Erwin, 1980). Resistance in soybeans to phytophthora root rot has been reported to be controlled by nine dominant genes (Layton et al., 1984). Twenty-four races of the pathogen have been identified (Keeling, 1984).

The plant introduction (PI) 437,833 was found to have resistance to brown stem rot (Rbs1) and other desirable agronomic traits when included in a plant introduction evaluation. Subsequent Pmg evaluation (unpublished data) revealed that PI 437,833 was resistant to race 1 of Pmg. The objective of this study was to determine the inheritance of resistance to Pmg in PI 437,833.

Materials and methods: Remnant F2 seed and progeny from individual F2 plants were evaluated in the greenhouse from the following crosses: 'Sherman' x PI 437,833, PI 437,833 x Century (Rps1^a).

Approximately 100 F2 seedlings from each cross to be tested were inoculated. Seedlings were grown in sand in the greenhouse for 10 days, then inoculated with Pmg race 1 zoospores, produced as described by Moots et al. (1983) at a concentration of 10^5 per ml, using the hypodermic inoculation technique (Schwenk et al., 1979). Five days after inoculation, seedlings were classified as resistant (no infection symptoms) or susceptible (hypocotyl collapse and death). The surviving F2 plants were then transplanted to the field. The following winter 20 seeds from 40 to 50 F2:3 families were grown in the greenhouse and inoculated with Pmg race 1. The data were analyzed using chi-square test for goodness of fit to expected ratios.

Results and discussion: The F2 population from the cross of susceptible line Sherman with PI 437,833 segregated in a ratio of 3 resistant:1 susceptible to race 1, which indicates that PI 437,833 has a single dominant gene for resistance to race 1 (Table 1). The F2:3 families were inoculated with Pmg race 1. The F2:3 families segregated in a ratio of 1 resistant:2 heterozygous. The F3 plants segregated in a ratio of 5 resistant:1 susceptible.

The F2 plants were all resistant in the cross involving Century carrying the Rsp1[a] gene with PI 437,833 when inoculated with Pmg race 1. This indicates that the gene controlling resistance to race 1 in PI 437,833 is allelic or is Rps1[a]. When F2:3 families were inoculated, 41 were resistant and 2 were heterozygous. There were a total of 2 out of 249 F3 plants that were susceptible. This could be due to an overload of zoospores in the system and should be overlooked.

Since PI 437,833 is susceptible to race 3 of Pmg, it is assumed that PI 437.833 has the Rps1[a] gene.

Table 1. Reaction of parents, F2, F3, and F2:3 families of the crosses PI 437,833 with soybean lines carrying the Rps1[a] and rps genes inoculated with race 1 of Phytophthora megasperma f. sp. glycinea.

Progeny	Generation	Reaction R[a]	H	S[a]	Ratio[b]	Chi square	P
Sherman x PI 437,833 rps	F2	99		34	3:1	0.0225	0.95-0.80
	F3	571		108	5:1	0.2654	0.80-0.50
	F2:3	18	32		1:2	0.1599	0.80-0.50
PI 437,833 x Century Rps1[a]	F2	144		0	R		
	F3	247		2	R		
	F2:3	41	2		R		
PI 437,833				109	0		
Century		24		0			
Sherman		1		45			

[a] R = resistant; S = susceptible.
[b] Ratio = resistant to susceptible, Ratio of 5:1 for F3 and 1:2 for F2:3 families due to eliminating susceptible F2 plants before transplanting to the field.

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1) Further genetic analysis of the unstable r-m allele.

The R gene of soybean is involved in the anthocyanin bio- synthetic pathway that is responsible for pigment accumulation in the seed coat. The r-m allele of this gene produces a striped seed coat with spots and/or concentric rings of black (dark purple) pigment superimposed on an otherwise brown seed coat. Previous reports have documented patterns of somatic and germinal instability for this particular allele that cause expression of the R locus to cycle between active and inactive phases (Chandlee and Vodkin, 1988, 1989). A summary of the genetic characteristics of the allele is as follows:

1) Somatic mutability of the r-m allele is demonstrated in those plants that produce mixtures of seed with different seed coat colors (i.e., black + striped or brown + striped).

2) Germinal mutability of the r-m is detected in plants producing all black or all brown seed. Progeny rows of the black-seeded plants either breed true or segregate 3:1 for black : striped. Progeny rows of the brown-seeded plants essentially breed true with few exceptions.

3) An unusual feature of the germinal revertants is that they continue to exhibit a low frequency of mutability back to the other allelic states of the gene. For instance, homozygous R*/R* black seed give rise to plants that produce progeny with the mixed seed phenotype (black + striped) or the striped-seed phenotype. Homozygous r*/r* brown seed can produce progeny with the striped-seed phenotype or the black-seed phenotype, but only on rare occasions.

The molecular mechanism responsible for the unstable nature of the r-m allele may involve large structural changes caused by insertions or deletions possibly due to transposable element activity; alternatively, subtle alterations caused by changes in DNA methylation patterns could lead to different levels of gene expression at the locus. The two explanations are not mutually exclusive, since methylation has been implicated in the regulation of transposable element activity (Chandler and Walbot, 1986; Masson et al., 1987). The tendency of the r-m allele to cycle between active and inactive phases resembles the behavior of various alleles found in corn that are known to have an associated transposable element and are regulated by methylation/demethylation events. It is with this in mind that this laboratory is approaching the molecular analysis of the r-m allele.

The most recent observations of the behavior of the r-m allele are as follows:

1) It has been reported that instability of the r-m allele can be induced through cross-hybridization (Chandlee and Vodkin, 1989) of different

soybean cultivars. In one cross, a resulting F_3 plant (designated F1484B1-1-1) produced a mixture of black, brown, and striped seed. Analysis of separate F_4 progeny rows revealed that the mutations represented strict somatic events and did not affect the germline except in one instance in which a plant produced all black seed. The progeny from this F_5 plant segregated 3:1 for black:striped seed coat color, indicating that the original plant was heterozygous R*/r-m. Analysis of single F_6 progeny rows led to the identification of plants exhibiting continued allelic instability. Two examples are: One F_6 progeny row resulting from an F_5 plant producing all striped F_6 seed generated plants with all black seed. It has not yet been determined if the original F_5 plant was homozygous R*/R* or heterozygous R*/r-m. A second F_6 progeny row resulting from an F_5 plant having all striped seed produced 59 plants with striped seed and one plant with black seed. Homozygosity or hetero- zygosity of the black seeded plant has not yet been determined. This analysis indicates that the r-m allele present in this cross, which was derived from a different genetic stock than the mutable stock, appears to maintain the capability for mutability for several generations.

2) A second hybridization between two other soybean cultivars resulted in an F_3 plant (designated F1884G2-4-1) with all black seed. The F_4 progeny row segregated 3:1 for black: striped, indicating that the original mutant was heterozygous R*/r-m. Analysis of single F_5 and F_6 progeny rows revealed continued instability of both alleles in subsequent generations. For instance, two F_4 plants that produced all black seed each gave rise to F_5 progeny rows that had all black-seeded plants. So, both plants appeared to be homozygous R*/R*. When the F_6 progeny rows were analyzed, reversion events back to r-m were detected since some of the progeny rows segregated 3:1 for black; striped. In a second instance, a single F_4 plant that produced all striped seed gave rise to F_5 progeny rows that had all striped-seeded plants, indicating that the original plant was homozygous r-m/r-m. However, in one F_6 progeny row, while the majority of the plants produced striped seed, two produced black seed. These two plants produced F_7 progeny that segregated 3:1 black:striped, indicating that they were heterozygous R*/r-m. These results indicate that the r-m allele can remain stable for several generations and then mutate to R*. So, again, for this particular cross, it appears that the potential for instability of the r-m allele can be maintained for several generations and that the mutation can occur from r-m to R* and vice versa.

3) Germinal revertants are commonly found to occur in four of the six possible directions, including r-m<-->R* (frequent) and r-m<-->r* (infrequent). The conversion of an R* allele directly to r* has not yet been observed and only one instance of change from r* directly to R* has been found. In addition, the r* allele appears to be much more stable than the R* allele. The R* allele frequently mutates back to r-m both somatically and germinally; however, r* almost never mutates back to r-m. The significance of this is not yet known. Examples of unstable r* alleles include one case in which it mutated to R* (RM-83-85-39-2) and another in which it mutated to r-m (RM-83-85-40-6, RM-83-85-40-7). The resulting R* and r-m alleles continue to exhibit mutability in subsequent generations. The r* allele, however, appears to remain stable in subsequent generations except in one instance in which it mutated to r-m again. Plans are underway to perform cross hybridizations in an attempt to induce instability of these stable r* alleles.

4) Southern blot analysis of DNA preparations from homozygous R*/R* and

homozygous r-m/r-m plants, using a Tgm1-specific 1.3 kb HindIII fragment as a probe, has revealed the presence of an additional 2.7 kb band in the R*/R* revertant when the genomic DNAs are cut with HaeIII/HhaI. The DNA preparations were made from individual plants of sublines derived from the mutable stock that were known to be true breeding for either of the respective phenotypes (striped seed or black seed) for at least three generations. In addition, other siblings in the progeny row were confirmed to be true breeding for the respective seed-coat phenotype. This type of care is necessary when choosing plants for analysis, due to the observed unstable nature of both the r-m and R* alleles. This result suggests that a genomic rearrangement or modification of a Tgm1-related sequence may be involved in the mutability observed for the r-m allele. The modification alternative (i.e., methylation of cytosine residues) seems plausible because the enzymes used for the blots both recognize GC-rich sequences. Also, the cycling behavior of the r-m allele suggests reversible modifications instead of gross chromosomal changes; however, both alternatives are still possible. It should be noted that the homozygous r*/r* plants show the same blot pattern as the r-m/r-m plants. Using the same care described above, crosses were made between homozygous stable r-m/r-m and R*/R* plants so that a cosegregation analysis of the blot patterns and the seed coat phenotype can be performed on the F2 progeny. This work is currently underway. If an association is found, it should provide a direct means by which the R locus and its allelic alternatives can be cloned to determine at the molecular level the mechanism resulting in the allelic instability of the r-m allele.

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Normal AL

IMPROVEMENT OF SOYBEAN FOR STRESS TOLERANCE AND BIOLOGICAL EFFICIENCY

1) Screening soybean genotypes for drought and heat tolerance.

Water deficits and high temperatures are among the most important environmental factors that limit crop productivity in many areas of the world. The lack of stability in soybean production because of variable climatic conditions has indicated a need to develop methods for improving this crop genetically through breeding cultivars capable of withstanding environmental stress.

Breeding for drought resistance has been accomplished by selecting for seed yield under field conditions (Bouslama and Schapaugh, 1984; Specht et al., 1986), but since such procedures require full season field data, it is not always an efficient approach, especially in mesic locations. An alternative may be to screen material under laboratory or greenhouse conditions, by using seeds or seedlings as test materials (Sammons et al., 1978). Several physiological characteristics in crops have been reported as being reliable indicators for the selection of plant germplasm possessing drought and heat tolerance. These characteristics include seed germination or seedling growth in hydroponic solution of low osmotic potential (Sammons et al., 1979; Stout et al., 1980; Sullivan, 1972) and heat tolerance measurement by the degree of electrolyte leakage from heat damaged leaf cells after exposure to elevated temperatures (Blum and Ebercon, 1981; Martineau et al., 1979; Sullivan and Ross, 1979).

The objectives of this study were to: 1) evaluate the genetic variability among several soybean genotypes subjected to drought and heat stress and 2) determine whether these parameters and other agronomic traits are effective criteria to select for drought and heat tolerance.

Several soybean genotypes, differing in growth habits from maturity groups III through VIII, were screened for drought and heat stress during germination and growth stages, respectively. The method used for drought test was the technique reported by Bouslama and Schapaugh (1984), whereas the technique used for measuring heat tolerance of leaf tissue was as described for sorghum (Sullivan, 1972), with minor modifications for soybean (Martineau et al., 1979).

Genetic variability was found among the lines for both drought and heat tolerance (Table 1). The germination stress index (GSI) ranged from a high of 89.34% for PI 393,550 to a low of 5.82% for PI 417,288, while zero percent was recorded for those genotypes that failed to germinate. In general, the large-seeded lines failed to germinate at 0.50 MPa. Among the 15 genotypes tested in 1988, heat injury ranged from a low of 30.65% in 'Williams 79' to a high of 63.80% in 'Jefferson'; while in 1989, for the additional 35 genotypes, it varied from 62.10% to 82.54% for PI 408,155 and PI 393,550, respectively.

A positive and highly significant relationship ($r = 0.98$) between

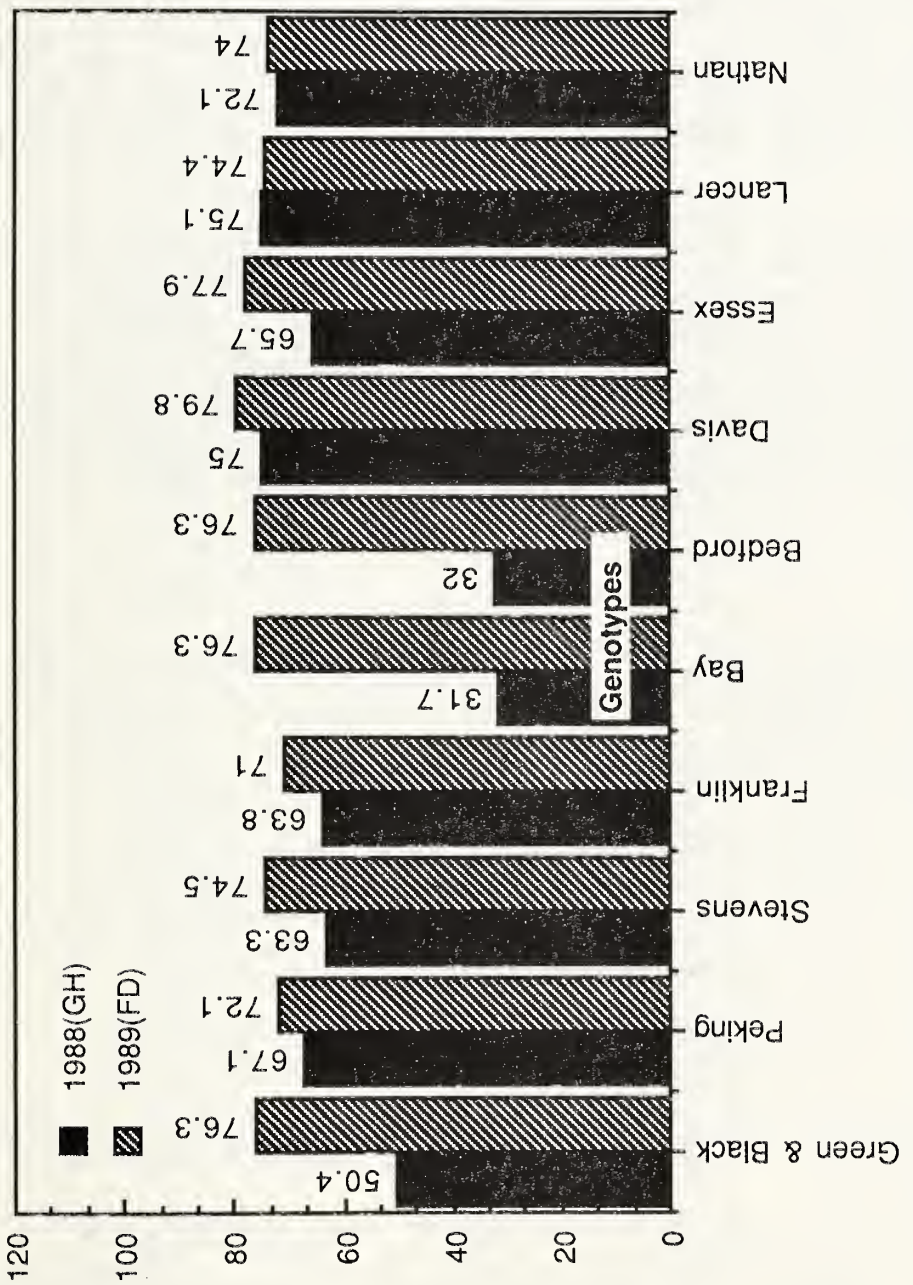

Fig.1 Percent heat injury of 10 soybean genotypes planted in the greenhouse and field in 1988 and 1989, respectively.

promptness index and GSI was found. However, negative correlation (r = -0.40) was observed between GSI and heat injury (Table 2). A highly significant and negative correlation (r = -0.74) between growth stages and heat injury was observed, indicating that heat injury decreased as growth stages advanced.

Ten genotypes that were common in our two-year study showed a very significant year effect and year x genotype interaction (Table 3.) Among few genotypes, a greater increase (e.g., 'Bay' and 'Bedford') or a smaller increase ('Nathan', 'Davis', and 'Peking') in injury was seen (Fig. 1). Thus, while percent injury increased from 1988 to 1989, this increase was large or small, depending on the genotype. The major difference between the two years in growth conditions was the very favorable water regime prior to and during the sampling period in 1989.

This study identified the lines PI 408,155, PI 423,827B, PI 423,759, and Pershing as both drought and heat tolerant. It is possible that the heat tolerance test could be used in soybean to select for drought tolerance, as was the case for corn, pasture grasses, and sorghum. However, not all genotypes that are heat tolerant appeared to be drought tolerant. On the basis of the results from the germination test, lines PI 393,547, PI 86,490, and PI 423,852 had high GSI (data not shown) and need further testing for heat stress. Those genotypes identified as both drought and heat tolerant are being studied further in the field and greenhouse in 1990.

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Table 1. Mean heat injury and germination stress index of 49 soybean genotypes.

Maturity group	Genotype	100-seed weight	Heat injury	Germination stress index
		1988		
		g	%	
III	Fuji	13.25	60.98	22.55
	Guelph	12.09	50.00	16.33
	Kim	24.81	51.73	0.00[a]
	Kanrich	24.0	49.37	0.00
	Columbia	13.52	47.10	33.85
	Wolverine	17.58	45.20	0.00
	Oakland	12.81	44.50	0.00
	Pella	15.46	36.63	0.00
	Williams 79	14.04	30.65	20.67
IV	Jefferson	26.68	63.80	0.00
	Emperor	22.06	62.83	0.00
	Imperial	28.55	58.67	0.00
	Ware	21.50	55.55	0.00
	Funk Delicious	23.34	40.65	0.00
	Sato	20.71	37.25	0.00
	LSD (0.05)		4.54	
		1989		
IV	Aodo	29.28	80.12	0.00
	RA 401	14.02	76.79	34.42
	Kailua	17.95	76.40	6.86
	Green & Black	21.20	76.34	38.16
	PI 82,264	7.10	75.63	64.85
	Sooty	9.70	75.99	38.75
	Kingston	8.00	75.60	31.82
	Shiro	23.40	74.79	0.00
	Norredo	8.30	74.62	54.66
	Stevens	13.46	74.54	34.38
	Peking	8.09	72.06	34.84
	Franklin	13.25	70.97	8.22
	Mid Summer	15.85	69.21	11.07
	PI 84,751	9.90	67.10	45.54
	Wilson 5	9.26	63.78	15.39
	Peking (Veg.)	9.00	63.01	31.37
V	PI 441.052[b]	8.27	78.84	75.95
	Essex	12.68	77.94	69.30
	Bay	17.35	76.34	17.81

Table 1. (continued)

Maturity group	Genotype	100-seed weight	Heat injury	Germination stress index
	Bedford	15.58	76.25	46.56
	PI 423,758	22.45	75.44	0.00
	PI 416,467	7.81	74.64	51.76
	PI 416,771	26.70	75.00	0.00
	PI 423,759	8.00	69.57	80.70
	Pershing	10.41	69.59	69.75
	PI 423,827B	6.65	68.96	74.06
	PI 417,288	23.60	63.56	5.82
	PI 408,155	8.20	62.10	81.01
VI	Davis	17.86	79.83	31.25
	PI 398,479	12.42	79.17	47.50
	Laredo	7.19	77.26	76.84
	Lancer	16.19	74.35	25.31
	Nathan	15.52	74.04	45.25
	PI 494,181	7.45	80.48	69.31
VII	PI 393,550	6.57	82.54[b]	89.34
	LSD (0.05)	1.67	4.63	3.53

[a] Zero percent was recorded for the genotypes that failed to germinate at -0.50 MPa.

[b] Final sampling for heat injury test was at R2 growth stage.

Table 2. Correlations among stress indices and other parameters in soybeans (1989).

	Promptness index	Plant height	Germination stress index	Heat injury
Seed weight	-0.79**	-0.54**	-0.84**	-0.44
Promptness index		0.98		
Germination stress index				-0.40
Growth stages				-0.74**
Plant height				0.41

** Significant at the 0.01 level of probability.

Table 3. Analyses of variance for variables measured in heat tolerance experiment.

Sources of variation	Degree of freedom	Mean squares
Replication	1	12.95
Years (Y)	1	2440.00**
Error A	1	0.39
Genotypes (G)	9	260.56**
Y x G	9	284.81**
Error	18	0.75
Total	39	--

** Significant at the 0.01 level of probability.

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2) 1988 and 1989 screening and selecting of vegetable soybean cultivars and germplasm for resistance to pests and diseases.

Collection of vegetable soybean cultivars and germplasm to provide resistant genes for diseases is essential. In order to improve biological efficiency of soybeans, traits such as resistance to diseases have to be investigated. The purpose of this study was to develop improved soybean germplasm for multiple resistance to pests and diseases.

Vegetable soybean cultivars and germplasm in maturity groups I - VIII were collected and planted in the field and were screened through natural infection of soybean stem canker (SSC), phytophthora root rot (PRR), bacterial blight of soybean (BBS), soybean mosaic virus (SMV), and soybean cyst nematode (SCN).

In 1988: 88, 85, 74, 83, 84, and 74 vegetable soybean cultivars and germplasm in Maturity Groups III - VIII were identified resistant to SSC, PRR, BBS, SCN, and SMV, respectively (Table 1). In 1989: 34 vegetable soybean cultivars and germplasm were likewise identified resistant to both SSC and PRR, and 8 and 11 were identified resistant to BBS and SMV, respectively (Table 2).

Those vegetable soybean cultivars and germplasm that were identified

with multiple resistance to pests and diseases will be further screened in controlled environment. The experiment is continuing.

Table 1. 1988 screening of vegetable soybean cultivars for multiple resistance to soybean diseases.

Soybean cultivars	MG[a]	Ratings of diseases[b]				
		SSC	BBS	PRR	SCN	SMV
1. Jogun	III	-[c]	-	-	-	-
2. Kanrich	III	-	-	-	-	-
3. Wolverine	III	-	-	-	-	-
4. Willomi	III	-	-	-	-	-
5. Oakland	III	-	-	+	-	-
6. Pella	III	-	-	-	-	-
7. Williams 79	III	-	-	+	-	-
8. Kim	III	-	-	-	-	-
9. Kura	III	-	+	-	-	-
10. Columbia	III	-	-	-	-	-
11. Fuji	III	-	-	-	-	-
12. Guelph	III	-	+	-	-	-
13. Emerald	IV	-	-	-	-	
14. Imperial	IV	-	-	-	-	-
15. Ware	IV	-	-	-	-	-
16. Emperor	IV	-	-	-	-	+
17. Funk Delic.	IV	-	-	-	-	+
18. Jefferson	IV	-	-	-	-	-
19. Sango	IV	-	-	-	-	-
20. Sato	IV	-	+	-	-	-
21. Aoda	IV	-	-	-	-	-
22. Green&Black	IV	-	-	-	-	-
23. Hahto/Mich.	IV	-	+	-	-	-
24. Kahala	IV	-	-	-	-	+
25. Kaikoo	IV	-	-	-	-	+
26. Kailua	IV	-	-	-	-	-
27. Mokapu (M) Summer	IV	-	-	-	-	-
28. Shiro	IV	-	-	-	-	-
29. Verde	IV	-	+	-	-	-
30. Kingston	IV	-	+	-	-	+
31. Norredo	IV	-	-	-	-	+
32. Peking	IV	-	-	-	-	-
33. Sooty	IV	-	-	-	-	-
34. Wilson	IV	-	-	-	-	+
35. PI 82,264	IV	-	+	-	-	+
36. PI 84,751	IV	-	+	-	-	-

Table 1. (continued)

Soybean cultivars	MG[a]	Ratings of diseases[b]				
		SSC	BBS	PRR	SCN	SMV
37. PI 85,505	IV	-	-	-	-	+
38. PI 86,103	IV	-	+	-	-	-
39. PI 339,984	IV	-	+	-	-	+
40. Pershing	IV	-	-	-	-	-
41. 5-931:408,155	V	-	-	-	-	-
42. 5-1035:416,771	V	-	-	-	-	-
43. 5-1083:PI416,982	V	-	-	-	-	-
44. 5-1096:417,05	V	-	-	-	-	-
45. 5-1120:PI417,159	V		No plant			
46. 5-1125:417,193	V	-	-	-	-	-
47. 5-1150:PI417,288	V	-	-	-	-	-
48. 5-1169:PI417,359	V	-	-	-	-	-
49. 5-1198:PI417,440	V	-	-	-	-	-
50. 5-1204:PI416,467	V	-	-	-	-	-
51. 5-2233:423,759	V	-	-	-	-	-
52. 5-1259:423,827B	V	-	-	-	-	-
53. 6-6:Hahto	VI	-	-	-	-	-
54. 6-8:Larredo	VI	-	-	-	-	-
55. 6-16:Rokusum	VI	-	-	-	-	+
56. 6-43:86490	VI	-	-	-	-	-
57. 6-86:171,437	VI	-	-	-	-	-
58. 6-136:222,397	VI	-	-	-	-	-
59. 6-188:398,479	VI	-	-	-	-	-
60. 6-190:398,479	VI	-	-	-	-	-
61. 6-331:PI417,213	VI	-	-	-	-	-
62. 6-340:417,310	VI	-	-	-	-	-
63. 6-374:423,852	VI	-	-	-	-	-
64. 6-485:494,181	VI	-	-	-	-	-
65. 7-105:PI181,565	VII	-	-	-	-	-
66. 7-114:337,687	VII	-	+	-	-	-
67. 7-262:393,550	VII	-	-	-	+	-
68. 7-265:393,550	VII	-	-	-	-	-
69. 7-274:PI416,893	VII	-	-	-	-	-
70. 7-275:416,900	VII	-	-	-	-	+
71. 7-288:PI417,128	VII	-	-	-	-	+
72. 8-47:181,697	VIII	-	-	-	-	-
73. 8-50:194,773	VIII	-	-	-	-	-
74. 8-169:324,068	VIII	-	-	-	-	+
75. Pershing:PV87		-	-	-	-	-
76. V-1232:PI423,758		-	-	-	-	
77. Stevens		-	-	-	-	-
78. Franklin		-	-	-	-	-
79. RA 401		-	-	-	-	-
80. Bay		-	-	-	-	-
81. Peking		-	-	-	-	-

Table 1. (continued)

Soybean cultivars	MG[a]	Ratings of diseases[b]				
		SSC	BBS	PRR	SCN	SMV
82. Bedford		-	-	-	-	+
83. Davis		-	-	-	-	-
84. Nathan		-	-	-	-	-
85. Essex		-	-	-	-	-
86. Lancer		-	-	-	-	-

[a] MG= Maturity Group, SSC=Soybean Stem Canker, BBS=Bacterial Blight of Soybean, PRR=Phytophthora Root Rot, SCN=Soybean Cyst Nematode, and SMV=Soybean Mosaic Virus.

[b] Ratings for diseases were as follows: 1-3 ratings=resistant with 0 to trace symptoms of diseases with less than 10% field infestation, 4-6 ratings=moderately resistant with 10-30% field infestation, and 7-9 ratings=susceptible with more than 30% disease field infestation (based on 1-9 disease scales where 1= no disease infection and 9=severe plant chlorosis, stunting, and complete defoliation or plants are dying or dead).

[c] =1-6 ratings, resistant; +=7-9 ratings, susceptible.

Table 2. 1989 screening of vegetable soybean cultivars for multiple resistance to soybean diseases.

Soybean cultivars	MG[a]	Ratings of diseases[b]			
		BBS	SMV	SSC	PRR
1. Vinton	I	+[c]	+	-	-
2. Magna	II	-	+	-	-
3. Price	II	+	+	-	-
4. Provar	II	+	+	-	-
5. Jogun	III	+	+	-	-
6. Kanrich	III	+	+	-	-
7. Wolverine	III	+	+	-	-
8. Willomi	III	+	+	-	-
9. Oakland	III	-	-	-	-
10. Pella	III	+	-	-	-
11. Williams 79	III	+	+	-	-
12. Kim	III	+	-	-	-
13. Kura	III	+	+	-	-
14. Columbia	III	+	+	-	-
15. Fuji	III	+	+	-	-
16. Guelph	III	+	+	-	-
17. Emerald	IV	+	+	-	-
18. Imperial	IV	-	+	-	-
19. Ware	IV	+	-	-	-
20. Emperor	IV	+	+	-	-
21. Funk Delicious	IV	+	+	-	-
22. Imperial	IV	-	-	-	-
23. Jefferson	IV	-	+	-	-
24. Sango	IV	+	+	-	-
25. Sato	IV	-	-	-	-
26. Aoda	IV	+	-	-	-
27. Green and Black	IV	-	+	-	-
28. Hahto/Michigan	IV	+	+	-	-
29. Kahala	IV	+	-	-	-
30. Kaikoo	IV	+	-	-	-
31. Kailua	IV	+	-	-	-
32. Mokapu (M) Summer	IV	+	+	-	-
33. Shiro	IV	+	-	-	-
34. Verde	IV	+	-	-	-

[a] Ratings for diseases are indicated in Table 1.
[b] Indicated in Table 1.
[c] Indicated in Table 1.

R. P. Pacumbaba
V. T. Sapra

3. Seed transmission of soybean mosaic virus using mottled seeds from virus-infected soybean plants.

Soybean mosaic (caused by soybean mosaic virus, SMV) is the most common virus disease of soybean and is a serious threat to soybean production in some areas of the United States (Cho and Goodman, 1979). Yield losses (Quiniones et al., 1971; Ross, 1969), reduced seed quality (Kennedy and Cooper, 1967; Ross, 1969), decreased oil content (Demski and Jellum, 1975), and decreased nodulation (Tu et al., 1970) have been reported in soybean as a result of SMV infection.

Soybean mosaic virus is vectored by aphids (Converse, 1948; Hill and Benner, 1980) and also transmitted through the seeds (Gardner and Kendrick, 1921; Hill and Benner, 1980). Seed coat mottling is associated with seed transmission of the virus (Ross, 1970; Sinclair and Backman, 1989). However, the relationship between mottling and virus transmission is inconsistent (Porto and Hagedorn, 1975) and unreliable of virus infection and presence of infective virus in seeds (Hill and Benner, 1980).

The purposes of this study were to 1) determine if all mottled seeds of soybean-induced SMV-infected soybeans carry the virus, 2) determine if only the SMV-infected plants induced mottled seeds or non-mottled seeds as well, and 3) determine how mottled seeds are produced and the chemical nature of the mottled seed coat.

Initially, 19 yellow soybean varieties were selected. One hundred mottled and 100 non-mottled seeds were planted in the greenhouse, replicated three times. The greenhouse temperature is maintained at 18-21 C. Four to six weeks after germination, the seedlings were scored for virus infection.

Initial results of six yellow-seeded soybean varieties tested for the transmission of SMV through mottled (black or brown) seeds (from SMV-infected plants) indicated that SMV and possibly peanut mottle virus (PMV) or cowpea chlorotic mottle virus (CCMV) and bean pod mottle virus (BPMV) were transmitted to the seedlings of 50.46% (based on the symptoms of infected seedlings). The rest of the seedlings from mottled seeds (49.81%) showed no symptoms of the viruses mentioned above. The non-mottled seeds (from supposedly SMV-free plants) transmitted SMV and possibly PMB/CCMV, and BPMV to the seedlings of 48.16%. The rest of the seedlings (51.85%) showed no symptoms of virus infection (Table 1). PMV, CCMV, and BPMV on soybean will be verified by serology test and also on the host range of these viruses. Other related experiments to satisfy the rest of the proposed objectives will be conducted at a later date. The experiments above are all continuing.

Table 1. Means of initial results of seed transmission of soybean mosaic virus, using mottled (black or brown) and non-mottled soybean seeds.[a]

Yellow seeded soybeans		No. of virus-infected plants SMV[b]	PMV/CCMV	BPMV	No. healthy plants	% diseased plants	% healthy plants
Bragg	M	46.33	16.33	15.33	6.67	93.60	8.01
	N	51.00	15.00	15.67	6.33	93.87	6.13
Franklin	M	17.67	7.50	6.67	37.83	45.70	54.30
	N	18.00	7.00	9.33	48.67	41.36	58.64
Hyper-farmer Smith B	M	21.00	16.00	7.00	29.00	60.28	39.73
	N	17.67	6.67	5.67	35.33	45.93	54.07
Pioneer 5482	M	14.66	7.67	5.67	30.67	47.72	52.28
	N	17.00	10.00	7.00	31.67	52.02	47.98
Funk 2910	M	10.33	10.33	3.67	21.33	53.28	46.72
	N	15.00	8.00	6.33	26.00	53.01	46.99
PI 9691	M	1.00	0.67	0.00	74.33	2.20	97.80
	N	2.00	0.00	0.00	71.00	2.74	97.26

[a] Data indicated are the means of three replications for each treatment per soybean variety.

[b] SMV=Soybean Mosaic Virus; PMV=Peanut Mottle Virus; CCMV=Cowpea Chlorotic Mottle Virus; and BPMV=Bean Pod Mottle Virus. M=Mottled (black or brown) seeds from soybean mosaic virus- infected plants and N=non-mottled seeds from supposedly healthy soybean plants.

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R. P. Pacumbaba

4) Effects of Al on the growth and symbiotic development of soybean germplasm.

The United States has become one of the largest soybean producing countries in the world and is now expected to compete with China and Japan in the production of specialty varieties of soybean (vegetable soybean) which can be used for manufacturing various soyfood products (Carter, 1987). These specialty varieties need to be screened for their tolerance to various environmental stress factors before they can be adapted successfully for growth in this country. One of the environmental stress factors, acid soil infertility, causes substantial reduction of soybean yield in certain parts of the USA. The combination of low pH and high Al concentration decreased the symbiotic development and overall plant growth in soybean and cowpeas (Alva et al., 1987; DeManzi and Cartwright, 1984; Hoihenberg and Munns, 1984). Alva et al. (1987) further noticed that the nodulation process in these plants was more sensitive to acidity and Al toxicity than the host plants themselves. Similar results have been found in tropical pasture legumes by Carvalho et al. (1981) and Whelan and Alexander (1986). They observed that even though Rhizobium trifolii grew well in a liquid medium with high Al (50 uM) at pH 4.5, in acid soil they could not nodulate their host plant significantly. This indicated that acidity and a high level of Al might have reduced the infection process in the roots, thus causing a substantial reduction in the nitrogen fixation process.

Soybean seeds of 50 selected soybean germplasms were surface sterilized and germinated in the dark for 3-4 days between filter papers. Bradyrhizobium strain USDA 110 and local isolate SM867 were transferred from agar slants to YEM (Yeast Extract Mannitol) broth and grown for 5-6 days in a slowly rotating shaker til they reached turbidity at approximate cell density of 10^8 cells per ml. Soybean seedlings were transferred to 4-liter plastic pots containing N-

Table 1. Plant growth response to varying Al levels in solution culture.

Al conc. uM	Root length** (cm)	Nod.no per plant	Nod.fr.wt. (g)	Shoot dry wt. (g)	ARA (umol C2H4/hr)	Total N (g/kg)
0	64.9a*	23.2a	0.19a	1.08	0.81a	28.9a
50	45.4b	9.3b	0.13b	0.77b	0.60b	25.6b
100	19.0c	5.6c	0.07c	0 59c	0.24c	23.8c

* Means with same letters in the column are not significantly different from each other at 5% probability level by DMRT.

** Values are per plant.

Table 2. Symbiotic performance of two Bradyrhizobium strains under varying levels of Aluminum.

Al conc.	Nod.no./pl.		Nod.fr.wt./pl		**ARA activity/pl		Total N in shoot (g/kg)	
	USDA110	SM867	USDA110	SM867	USDA110	SM867	USDA110	SM867
0 uM	20.7a*	24.8a	0.18a	0.21a	0.77a	0.81a	29.1a	28.8a
50 uM	8.5b	10.3b	0.12b	0.14b	0.63b	0.49b	25.5b	25.7b
100 uM	5.3b	5.8b	0.06c	0.09c	0.26c	0.21c	23.2b	24.3b

* Means with same letters in the column are not significantly different from each other at 5% probability level using DMRT.

** umole C2H2/hour.

Table 3. A comparison of the performance of selected soybean germplasm at three levels of Al.

Soybean Germplasm	Nodule numbers/plant (Al concentrations)			Nodule fresh weight (g/plant) (Al concentrations)			**ARA activity/plant (Al concentrations)		
	0 nM	50 uM	100 uM	0 uM	50 uM	100 uM	0 uM	50 uM	100 uM
Kim	36.a*	12.1d-j	11.0e-j	0.28abc	0.23a-f	0.12c-j	1.5abc	0.73c-h	0.27d-h
Wolverine	33.1ab	17.0c-i	6.9g-j	0.20a-g	0.26a-d	0.15c-j	0.78b-h	0.95a-f	0.47d-h
Kahala	27.6abc	8.1g-j	3.6hij	0.32a	0.19a-h	0.11d-j	1.53ab	1.69a	0.29d-g
Kailu	25.3a-d	7.5g-j	4.3hij	0.31a	0.14c-j	0.09d-j	0.78b-h	0.36d-h	0.26d-h
Oakland	24.8a-d	11.4e-j	6.0g-j	0.18a-h	0.08f-i	0.05g-j	0.42d-h	0.19e-h	0.20d-h
Fuji	23.4b-e	9.7f-j	4.2hij	0.16b-j	0.08f-j	0.02ij	0.79b-h	0.43d-h	0.17e-g
Kaikoo	22.2b-f	9.4f-j	3.9hij	0.18a-i	0.18a-i	0.05g-j	0.96a-f	0.99-e	0.38d-h

* Means with same letters in the column are not significantly different from each other at the 5% probability using DMRT.
** umoleC2H4/hour.

free nutrient solution (Franco and Munns, 1982) with 0, 50, and 100 uM Al added as $AlK(SO_4)$. 12 H_2O and inoculated with 10 ml of rhizobia broth. Plants were continuously aerated and grown for 35 days in a growth chamber at 27 C for 16 h light and 20 C for 8 h dark period. The pH was adjusted to 4.5 daily using 1N HCl or 1N KOH. After harvesting, root lengths were measured, nodule numbers counted, and ARA activity measured by using a Perkin Elmer Sigma 2000 gas chromatograph. Total nitrogen in shoots was determined by using a Kjeltec nitrogen analyzer.

Plant growth: Soybean germplasms 'Kingston', 'Norredo', 'Sooty', and 'Wilson 5' were extremely acid sensitive. Almost all plants died in the nutrient solutions after two weeks. In general, higher concentration of Al in the growth medium severely affected the initial growth of the plants. The Al toxicity was mostly visible in the root growth of the plants, where root lengths were severely stunted at both 50 and 100 uM Al. Root lengths were significantly reduced in each germplasm line as the Al concentration increased 0 to 100 uM. At harvest (35 days), there were no significant differences in primary root lengths between 0 and 50 uM Al, but overall plant growth was significantly reduced at Al concentration of 100 uM. Table 1 shows that there was an overall reduction in plant growth, e.g., root lengths, shoot dry weight, nodule numbers and weight and total N in the shoots as the Al concentration in the nutrient solution increased from 0 to 50 to 100 uM. Appearance of the first nodule in the control plants (0 uM Al) occurred around 6-7 days. They were delayed for 2-3 days when the concentration of Al increased to 50 uM and there was a further delay of 4-5 days at the 100 uM Al level in the growth solution.

Symbiotic development: No significant differences in symbiotic development were observed by inoculating the plants either with Bradyrhizobium strain USDA 110 or the local isolate SM867. This might be because both of these strains showed tolerance to low pH and high Al from previous screening studies. But within each strain there was a significant reduction in nodule numbers, nodule fresh weight and ARA activity as the Al concentrations in the growth solution increased from 0 to 100 uM (Table 2). In most germplasm lines, 50 uM Al in the growth solution increased the size of the nodules. This is the reason why there were no significant differences in ARA activity between plants grown in 0 uM Al and 50 uM Al (Table 3). Germplasm line 'Kim' had the highest numbers of nodules at the 100 uM Al concentration, but 'Kahala' had the highest ARA activity at 0 and 50 uM Al (Table 3). In general, germplasm lines that tolerated pH 4.5 were also more tolerant to 50 uM Al in the growth solution.

Conclusion: Symptoms of Al toxicity at both 50 and 100 uM Al were more visible in the root zone of all soybean germplasm lines where root lengths were severely stunted in the first weeks of the plant growth. In some germplasm lines, plants grown in the 50 uM Al overcame the toxicity symptoms after the third week and there were no significant differences in their lateral root lengths between 0 and 50 uM Al. At both 50 and 100 uM concentrations of Al, the symbiotic development of all the germplasm lines was significantly reduced. There was a significant reduction in nodule numbers and ARA activity at higher concentrations of Al. Germplasm lines 'Kahala', 'Kaikoo', 'Wolverine', and Kim showed more tolerance to 50 uM Al than other lines. Kim and Wolverine were both tolerant to 100 uM Al compared with other germplasm lines screened in this study.

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5) Responses of various soybean genotypes to excess soil manganese.

Introduction: Manganese toxicity is one of the major growth-limiting factors in many acid soils having pH values of 5.5 or below, if the soil parent materials contain sufficient total Mn (Foy, 1973, 1981). Plant species and cultivars differ widely in their tolerance to excess soluble or exchangeable Mn (Foy, 1983). Corn and rice are more tolerant than soybean or barley. Excess Mn affects plant tops more directly than roots (Foy, 1973, 1982a). For a given plant, Mn accumulates somewhat in proportion to plant injury (Osawa and Ikeda, 1980). The selection of genotypes tolerant to manganese toxicity in acid soils may significantly affect the seed yield of soybean (Boswell et al., 1981). The objective of this study was to determine the sensitivity or tolerance of various vegetable soybean genotypes to high levels of soil manganese and low soil pH.

Materials and methods: An Enon sandy loam soil was used for the study in a greenhouse. The soil was analyzed with respect to texture, pH, cation exchange capacity (CEC), organic matter and extractable Mn. The soil was divided into two batches, and one batch was adjusted to pH 4.8 and the other to pH 6.3 with 3N HCl and $CaCO_3$. The soil was allowed to equilibrate for a few weeks to stabilize the pH level and determined. Then, soil was placed in 15-cm diameter plastic pots and 1 g per pot of 5-10-10 N-P-K fertilizer applied to provide the major nutrients. A split plot design with three replications of each treatment was used. pH levels of the soil were the plot treatments and soybean genotypes were subplot treatments. Fifty three vegetable soybean genotypes belonging to different maturity groups (III to VIII) were tested in the study.

At initiation of flowering, plants were harvested and their dry weights recorded. Also, fully developed leaves were collected for Mn analysis. The data obtained were subjected to statistical analysis by using analysis of variance procedure.

Results and discussion: Genotypes PI 423,827, PI 417,440, PI 393,550, PI 417,322, PI 171,437, PI 423,758, 'Wilson 5', 'Guelph', 'Rockusun', 'Laredo', 'Kura', 'Sango' and some others were very tolerant to high levels of soil Mn and low soil pH, and their shoot weights were unaffected under the conditions (Table 1). Genotypes PI 423,852, PI 339,984, PI 82,264, PI 417,128, 'Hahto', 'Kanrich', 'Sooty', 'Columbia' and some others were sensitive to high levels of soil Mn and low soil pH, and their shoot weights decreased at pH 4.8. According to analysis of variance, there was a significant difference ($P<0.01$) in shoot weights between the genotypes. Overall, the pH effect was significant ($P<0.01$).

In general, Mn concentration was much higher in the soybean leaf tissue at pH 4.8, compared with pH 6.3. The genotypes that were sensitive to Mn did not tolerate the high level of Mn in the plant tissue, showed Mn toxicity symptoms and resulted in reduced shoot weight at pH 4.8. The genotypes with

high concentration of Mn in leaf tissue and unaffected shoot weights at pH 4.8 appear to tolerate excess Mn. In summary, the genotypes found to be tolerant to Mn toxicity could be utilized in breeding programs for development of better vegetable soybean cultivars for problem soils.

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Table 1. Vegetable soybean genotypes sensitive or tolerant to high soil manganese and low soil pH reflecting in shoot weight differences.

Genotypes	Soil pH 4.8 (High soil Mn)	Soil pH 6.3 (Low soil Mn)	TI* Index
PI 423,827	1.85	0.61	303
Wilson 5	3.15	1.26	250
Guelph	2.87	1.72	167
PI 417,440	3.50	2.18	161
PI 393,550	2.95	1.99	148
PI 417,322	4.73	3.26	145
PI 171,437	3.00	2.19	137
Rokusun	2.88	2.10	137
PI 423,758	3.00	2.27	132
PI 86,490	2.53	1.98	128
PI 417,213	3.67	2.92	126

Table 1 (continued)

Genotypes	Soil pH 4.8 (High soil Mn)	Soil pH 6.3 (Low soil Mn)	TI* Index
PI 416,900	2.97	2.37	125
PI 230,972	2.96	2.70	110
Laredo	2.71	2.46	110
Kura	4.10	3.77	109
PI 86,103	2.87	2.66	108
PI 84,751	2.67	2.50	107
Sango	3.42	3.21	107
PI 417,052	2.25	2.12	106
PI 398,479	2.75	2.59	106
Norredo	2.73	2.61	105
PI 417,288	3.38	3.31	102
Aoda	3.05	3.03	101
PI 393,547	3.01	3.00	100
PI 194,773	2.90	2.97	98
Emerald	3.57	3.63	98
PI 417,159	3.13	3.20	98
PI 408,155	2.50	2.58	97
Hahto/Michigan	3.03	3.12	97
Sato	3.18	3.29	97
Kim	3.43	3.55	97
PI 494,181	2.30	2.40	96
PI 417,193	1.89	2.04	93
Funk Delicious	3.57	3.89	92
Kahala	2.90	3.17	91
PI 65,926	2.03	2.27	89
PI 181,697	2.70	3.04	89
PI 85,505	2.28	2.56	89
Peking	2.23	2.51	87
Kingston	2.43	2.79	87
Fuji	2.80	3.24	86
Verde	3.32	3.87	86
Shiro	3.10	3.59	86
Mokapu summer	2.50	2.94	85
PI 423,759	2.34	2.95	82
PI 417,128	2.22	3.13	80
Columbia	2.43	3.00	78
Sooty	2.50	2.93	76
PI 82,264	2.22	3.06	73
Kanrich	2.83	4.04	70
PI 339,984	2.42	3.56	68
PI 423,852	2.47	4.00	62
Hahto	2.45	4.78	51

* Tolerance Index >100 very tolerant, 89-99 tolerant, 81-88 sensitive, 51-80 very sensitive.

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